Management of Potato Leafhopper in Alfalfa

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December 2023

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

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LIST OF ABBREVIATIONS

PLH – Potato leafhopper

CP – Crude protein

NDF – Neutral detergent fiber

ADF – Acid detergent fiber

SAB – Sainte-Anne-de-Bellevue

POC – La Pocatière

g – Gram

kg – Kilogram

Mg – Milligram

ha – Hectare

ABSTRACT

The potato leafhopper [PLH, *Empoasca fabae* (Harris)], which affects several crops including alfalfa (*Medicago sativa* L.), is a recurrent problem in several regions of Quebec. The objective of the current project was to evaluate different management tools in order to reduce negative effects locally caused by this pest in alfalfa fields. Two experiments were conducted at two sites in Quebec over three field seasons to evaluate the effects of insecticide applications, the use of PLH-tolerant cultivars, and mixtures with grasses on PLH populations, forage yield, and forage nutritive value. Foliar insecticide applications in the seeding year reduced PLH populations but generally failed to affect alfalfa yields compared to alfalfa not treated with insecticides. However, in both experiments at one site, applications done in the seeding year had an indirect residual effect increasing alfalfa yields in early harvests of the first post-seeding year, in low PLH conditions, compared to alfalfa not treated with insecticide. Harvesting also effectively reduced PLH populations. The use of PLH-tolerant cultivars provided limited benefits with high PLH populations across environments and some yielded less than susceptible cultivars with low PLH populations in some environments. Additionally, mixing alfalfa with grasses overall had limited impact on the alfalfa-PLH interaction. Our results suggest that foliar insecticide applications and earlier harvests could be more effective ways to reduce PLH populations and their effect on alfalfa than using PLH-tolerant cultivars. Results will, however, need to be confirmed in a greater number of environments.

RÉSUMÉ

La cicadelle de la pomme de terre [CPT, *Empoasca fabae* (Harris)], qui affecte plusieurs cultures dont la luzerne (*Medicago sativa* L.), constitue un problème récurrent dans plusieurs régions du Québec. L'objectif du présent projet était d'évaluer différents outils de gestion afin de réduire les effets négatifs causés localement par ce ravageur dans les champs de luzerne. Deux expériences ont été menées à deux sites au Québec pendant trois saisons pour évaluer les effets d'applications d'insecticides, de l'utilisation de cultivars tolérants à la CPT et de mélanges avec des graminées sur les populations de CPT, le rendement fourrager et la valeur nutritive du fourrage. Les applications foliaires d'insecticides au cours de l'année du semis ont réduit les populations de CPT, mais n'ont généralement pas eu d'effet sur les rendements de luzerne par rapport à la luzerne non traitée. Cependant, dans les deux expériences réalisées à un site, les applications effectuées au cours de l'année du semis ont eu un effet résiduel indirect augmentant les rendements de luzerne lors des premières récoltes de la première année suivant le semis, dans des conditions de faibles populations de CPT, par rapport à la luzerne non traitée. Les récoltes ont également effectivement réduit les populations de CPT. L'utilisation de cultivars tolérants à la CPT a offert des avantages limités avec des populations élevées de CPT dans tous les environnements et certains ont produit des rendements inférieurs à ceux des cultivars sensibles avec une faible population de CPT dans certains environnements. L'ajout de graminées à la luzerne dans l'ensemble a eu un impact limité sur l'interaction luzerne-CPT. Nos résultats suggèrent que les applications foliaires d'insecticides et des récoltes plus précoces pourraient être des moyens plus

efficaces de réduire les populations de CPT et leurs effets sur la luzerne que l'utilisation de cultivars tolérants à la CPT. Les résultats devront cependant être confirmés dans un plus grand nombre d’environnements.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my supervisor, Dr. Philippe Seguin, for giving me this opportunity to work on this project under his supervision. I am very grateful for his invaluable teaching and guidance for all the field and lab work, data analyses, thesis writing and editing, as well as conferences presentations. I would also like to thank him for his constant support and encouragement throughout the past two years.

I would like to thank research assistant Mr. Parghat Gopal, for patiently teaching me how to conduct nutritive analyses in the laboratory and working skills at the farm, as well as guiding and supporting me whenever I needed some help.

I would like to thank my committee member Dr. Valerie Gravel, for giving me advice and guidance for my experiments and presentations. I would like to thank Mr. Marc Samoisette, for giving me technical support and guidance at the farm. I would like to thank all my lab mates and summer students for helping me with my harvests and data collections in Sainte-Anne-de-Bellevue, particularly, Juliette Keith, Ximena Florez Buitrago, Shane Liu, Alice Samoisette, Palaniappan Ramanathan, and Yuxuan Tang. I would also like to thank all the researchers and technicians of the Centre de Développement Bioalimentaire du Québec Inc (CDBQ) and the Centre de recherche sur les grains (CEROM) whom were involved at the La Pocatière site, including Julien Saguez, Benjamin Mercier, Vincent Banville, and Céline Georlette, for taking care of our project over there.

I would like to thank the financial support for my project from the Innov'Action program, a program from the Canada-Quebec Agreement for the Implementation of the Canadian Agricultural Partnership between the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation and Agriculture and Agri-Food Canada.

Finally, I would like to give a big thank to all my friends, my brother and my parents for their continuous warm encouragement, motivation, and support throughout my life.

CONTRIBUTION OF AUTHORS

This thesis has been written following the “Guidelines Concerning Thesis Preparation” of McGill University and is the effort of a collaboration primarily between McGill University, the Centre de Développement Bioalimentaire du Québec Inc (CDBQ), the Centre de recherche sur les grains (CEROM), the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ), and Agriculture and Agri-Food Canada (AAFC). Sections 3.2 and 4.2 (Materials and methods) were initially developed by Philippe Seguin (McGill), Céline Georlette (CDBQ), Huguette Martel (MAPAQ), and Julien Saguez (CEROM), with input from Annie Claessens (AAFC). The field work presented in Sections 3.2.2 and 4.2.2, were done in most parts by Xiawei Shi and supervised by P. Seguin in Sainte-Anne-de-Bellevue and by J. Saguez and his team in La Pocatière. Lab work and statistical analyses presented in Sections 3.2.3 and 3.2.4, was done by X. Shi and supervised by P. Seguin. The results, discussion, and conclusions sections of Chapter 3, 4 and 5 were primarily written by X. Shi and revised by P. Seguin. Modified versions of Chapters 3 and 4 of the current thesis will eventually be submitted for publications in peer-reviewed journals with researchers involved the development and realization of this project as co-authors.

CHAPTER 1

GENERAL INTRODUCTION

Canada is a world-leading agricultural production country (Sarkar et al., 2018). Ontario and Quebec account for 73% of the dairy production, therefore forages are locally considered as major crops (McCartney & Horton, 1997). Among perennial forage species grown, alfalfa (*Medicago sativa* L.) is the main crop and thus is called the "Queen of Forages", being grown on about 3.7 million hectares in Canada (Barnes et al., 1988; Jing et al., 2020; Statistics Canada, 2023). Alfalfa is a perennial flowering plant from the Fabaceae family which is not only important in North America but throughout the world, being produced on about 30 million hectares worldwide (Singer et al., 2018; Pomerleau-Lacasse et al., 2019; Jing et al., 2020). In addition to being used as forage crop, alfalfa, has several other potential use and environmental benefits, such as fixing atmospheric nitrogen and sequestering organic carbon in the soil, which might have the potential to help slow down global warming (Jing et al., 2020). However, the growth of this legume is subject to biotic and abiotic stresses, the frequency and intensity of which are increasing due to climate change, including increased temperatures, increased occurrence of drought, and changes in snow accumulation during the winter (Singer et al., 2018). Climate change could also generate new biotic stress conditions. For example, alfalfa producers in North America see a change in the prevalence of certain pests, including insects, bacteria, fungi, and viruses, which can also affect alfalfa production and thus affect the profitability of dairy production; alfalfa being a key component of cows ration (Suzuki et al., 2014).

The potato leafhopper [PLH; *Empoasca fabae* (Harris); Homoptera: Cicadellidae], is an insect native to North America, which is a serious pest affecting several key crop species in the USA and Canada (Chasen et al., 2014). Potato leafhoppers have a variety of host plants, up to 200, including alfalfa, soybean, potato, and peanut (Bullas Appleton et al., 2003). It migrates from the southern United States to northern United States and Canada each summer. This insect does not overwinter in Canada as it is killed by low temperatures (Chasen et al., 2014). The potato leafhopper is a major economic pest of alfalfa. It was reported that alfalfa crop losses induced by this insect in Maryland represented as much as $66/ha in 1987, while in Ontario, alfalfa production losses were estimated to be of about 183 million dollars in 2001 (Lamp et al., 1991; Bullas Appleton et al., 2003). Potato leafhopper feeding on alfalfa results in plant height and biomass reduction, thus decreasing forage yields, which contributes to major economic loss to alfalfa (Calvin et al., 2013). Furthermore, potato leafhopper feeding changes the chemical composition of alfalfa, potentially affecting the concentration of crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF), as well as *in vitro* digestible dry matter (IVDDM) (Sulc et al., 2004; Sulc et al., 2015). The impact of potato leafhopper increases significantly in dry, hot conditions (Chasen et al., 2014). Although potato leafhopper is now an endemic pest in the USA (Sulc et al., 2004), only in recent years it has become an emerging problem in Quebec. As this insect has only been reported in some regions locally for the first time in the past 10 years, there is thus very limited information on its impact on alfalfa production in Quebec. This project aimed at quantifying the losses associated with potato leafhopper in Quebec and evaluate pest management methods in alfalfa that includes insecticide applications, the use of tolerant cultivars and alfalfa-grass mixtures.

1.1. Objectives

(1) Quantify the effects of potato leafhopper on alfalfa in field conditions of Quebec and assess differences in susceptibility among a range of susceptible and tolerant cultivars.

(2) Evaluate different management strategies to reduce the impact of potato leafhopper on alfalfa by identifying the most tolerant cultivars, quantifying the effect of insecticides, and assessing the potential of using mixtures with grasses to reduce impact.

1.2. Hypotheses

(1) Potato leafhoppers negatively impact forage yield and nutritive value of alfalfa.

(2) Potato leafhopper tolerant alfalfa cultivars are less impacted by potato leafhopper and their use can reduce the need for insecticides.

(3) Mixing grasses with alfalfa reduces potato leafhopper injury in alfalfa, which in turn will increase total forage yields compared to pure alfalfa stands.

CHAPTER 2

REVIEW OF LITERATURE

2.1. GENERAL BACKGROUND

Agriculture is the third largest industry in Canada after mining and oil, although only 7% of land is used for agriculture (68 million ha), of which more than 36 million ha are used for livestock grazing and forage crop production (McCartney & Horton, 1997). The dairy industry is an important part of Canadian agriculture, with 1.8 million dairy cows, more than 70% of which are in Quebec and Ontario, creating a forage-based livestock industry (Statistics Canada, 2017; Statistics Canada, 2023). Forages are a fundamental part of the dairy industry being the largest component of their ration, therefore they are critical to the sustainable economic development of dairy production in Canada (Martin et al., 2017). It is evident that improving the nutritional value, yield and persistence of forage crops can help improve the potential profitability of the dairy industry (Claessens & Biligetu, 2018). Alfalfa (*Medicago sativa* L.), one of the main perennial forage crops used in Canada, is often grown pure or in mixtures with other forage species. Alfalfa is considered a sustainable crop because it can biologically fix nitrogen from the atmosphere, reducing the need for industrial nitrogen fertilizer in agroecosystems. With its high yield and high nutritional value, it is no surprise that alfalfa is known as the "Queen of forages" (Jing et al., 2020). However, alfalfa production faces various challenges, including both abiotic and biotic stress, such as climate change, salinization, disease, and insect predation (Singer et al., 2018; Yan et al., 2023). The potato leafhopper [*Empoasca fabae* (Harris)], a major pest in alfalfa across the eastern US and parts of Canada, can exert a negative effect on the yield, chemical compositions, and nutritional value of alfalfa (Sulc et al., 2004). In the past ten years, this legume has been found to be increasingly affected by potato leafhoppers in Quebec, to become a severe problem in some regions. There is, however, limited information regarding the effect of potato leafhoppers on alfalfa production in Quebec.

2.2. ALFALFA

Alfalfa, along with soybean (*Glycine max* (L.) Merr.) and lentil (*Lens culinaris*), belongs to the legume family, the former having a long history of being used as a forage crop. There are numerous benefits to grow alfalfa. As a leguminous plant, alfalfa forms a symbiotic relationship with a species of rhizobium (*Sinorhizobium meliloti*) that helps it fix nitrogen (Wang et al., 2021). Since alfalfa is in a different family from grass crops [such as corn (*Zea mays*) and oat (*Avena sativa*)], crop rotation can reduce the incidence of diseases and parasites in these crops (Yost et al., 2020). As a forage crop, alfalfa is high in yield and nutritive value, containing high protein concentrations and a variety of vitamins and minerals (Kumar, 2011; Liang et al., 2019). Alfalfa is considered low in fiber content but high in protein (15-22% Crude Protein, CP) compared to other forage crops, thus greatly reducing the need for extra costly protein supplements in livestock rations (Kumar, 2011; Chasen et al., 2014). In Quebec and Ontario, which have major dairy production, 18% of the land area in the two provinces is devoted to alfalfa production (McCartney & Horton, 1997; Wang et al., 2021). Although alfalfa is a hardy perennial plant with a deep root system that helps prevent soil erosion in arid lands, harsh environmental factors, including high salt and drought stresses, can cause significant reductions in alfalfa productivity (Li et al., 2014; Liang et al., 2019).

Although alfalfa is widely grown in Quebec, it needs specific soil types and conditions suitable for its growth. Well drained and fertile soil conditions are most appropriate. It grows best in soils with pH between 6.6 and 7, being poorly adapted to low pH conditions. Fertilization is another critical factor for growing alfalfa. As other species it does need nitrogen to grow, though to a lesser extent, as this nutrient can be fixed by rhizobia once nodules are formed. In addition, P and K are crucial elements, P can promote the growth of the root system, and K can enhance the winter survival of alfalfa (Undersander et al., 2011).

2.3. POTATO LEAFHOPPER

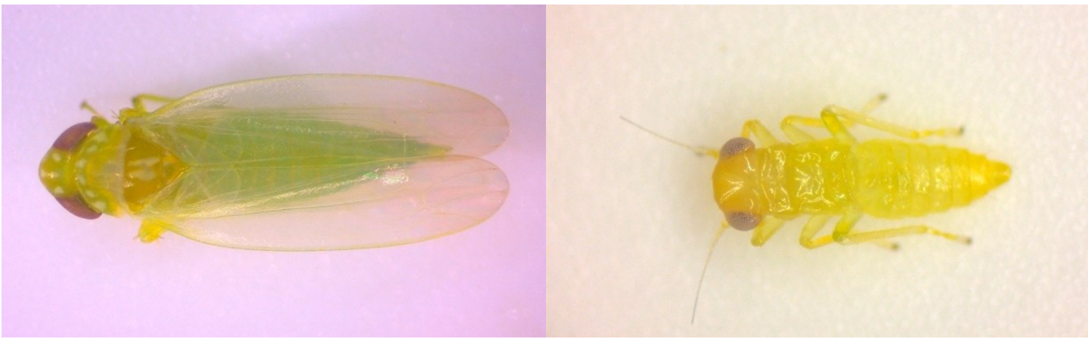

Photo 2.1. Potato leafhopper adult (left) and nymph (right).

The potato leafhopper is an insect that feed on the sap of several plant species which has long been considered an important pest of several crop species as it can cause significant yield losses (Nielsen et al., 1990; Delay et al., 2012). The potato leafhopper is a migratory insect that migrates from the southern United States to Canada each spring. It never overwinters in Canada, thus infestation levels in a year cannot be used to predict it in subsequent years. Besides, the environmental conditions including weather and temperature also affect the arrival time and growth rate of the potato leafhopper once it establishes in a specific region (Hansen et al., 2002). An adult lays 2 to 5 fertile eggs per day on the stem or large leaf vein, and each adult can lay up to 200 eggs (Chasen et al., 2014). Besides, it takes around 2 weeks or not more than 4 weeks to develop from egg to adult, depending on the temperature conditions. Therefore, the life cycle of the potato leafhopper is extremely fast and thus potato leafhopper populations can soar in a brief period.

There are about 220 species of plants that can act as host in 26 families, among which alfalfa is one of the main crop species. The typical symptom of potato leafhopper attack on alfalfa is called hopperburn. This symptom begins as a yellow “V” shape that radiates from the center to the tip of the leaf. Potato leafhoppers feed through the vascular tissues of plants, which cause the xylem cells to block, thus reducing photosynthesis and transpiration rates, and ultimately the transport of photosynthetic compounds and the accumulation of starch in leaves (Delay et al., 2012; Chasen et al., 2014). Injury from potato leafhoppers can also cause stem elongation to stop and premature leaf abscission (Hansen et al., 2002). The presence of potato leafhoppers in alfalfa fields can cause decreases of 13-27% in forage yield and 3-39% in crude protein (CP), as well as significant reductions in the concentration of several nutrients, including vitamin A, carotene, calcium, phosphorus, and digestible dry matter (Nielsen et al., 1990; Sulc et al., 2004). It is thus important to control potato leafhopper populations in alfalfa fields. Furthermore, the climatic conditions that favor the reproduction of leafhoppers are warm temperatures and drought, which correspond to the conditions more frequently observed in recent years and which could intensify with projected climate changes.

2.3.1. Insecticide application

Foliar insecticides have been considered an effective way to control potato leafhopper nymphs and adults in several crops (Oloumi-Sadeghi et al., 1989). Pyrethroids, organophosphates, neonicotinoids, or mixtures of these are commonly applied for the control of potato leafhopper nymphs and adults in alfalfa fields, and commercial formulations of dimethoate (Cygon 480), flupyradifurone (Sivanto Prime) and cyhalothrin-lambda (Matador) are some of the effective insecticides that have been used in recent years to successfully reduce insect populations in alfalfa fields (Chasen et al., 2014; CRAAQ, 2023; Lai & Nault, 2023). Currently, the management strategy is based on monitoring potato leafhopper populations throughout the growing season and treat with insecticides when insect populations reach the economic threshold (DeGooyer et al., 1998; Chasen et al., 2014). The threshold values developed in the American Midwest and depend on alfalfa plant height, for example, 0.4 leafhoppers per net sweep when the height is less than 15 cm, 0.8 when the height is 20-24 cm, 1.2 when the height is 30-34 cm, etc… (RAP 2020). One of the problems with using insecticides to control potato leafhoppers is that some of the insecticides used have relatively high health and environmental impact indices and can kill beneficial insects such as bees (CRAAQ, 2023). Currently, depending on the insect populations, the region and year, even with scouting, two to three applications per season may be necessary. A reduction in the use of insecticides would therefore be desirable. In addition, since the living habit of potato leafhopper varies depending on the weather and environmental conditions, it would be warranted to evaluate the thresholds which were developed in the USA in Quebec (Chasen et al., 2014).

2.3.2. Glandular-haired alfalfa cultivars

For alfalfa, the control of potato leafhoppers relied mostly on the use of insecticides combined with scouting until glandular-haired alfalfa cultivars, which are tolerant to potato leafhoppers, were released in 1997 (Ranger & Hower, 2001). Tolerance to potato leafhoppers comes from physical properties, namely glandular hairs, which ooze tiny droplets of sticky materials that can block the movement of both adult and nymph potato leafhoppers, reducing their feeding rate and egg production (Obermeyer, 2020). Many of alfalfa cultivars originally developed were more suited to the Midwest US, but cultivars adapted to conditions in the Eastern US and Canada have since become available, including some in Quebec from local seed companies (e.g., Synagri, Pickseed, Pioneer). However, to our knowledge, no study has evaluated these different cultivars in the same comparative trial, and their performance compared to that of susceptible traditional cultivars is not known. In the United States, various studies carried out in the North suggest that the performance of cultivars tolerant to potato leafhoppers varies according to cultivars and trial sites. For instance, yield gains of 50% have been reported in the presence of high potato leafhopper populations by Sulc et al. (2001) in a study carried out in the Midwest US comparing the yields of cultivars tolerant and susceptible to potato leafhoppers. In contrast, only one year of four in a trial in New York State showed higher forage yields of potato leafhopper-tolerant alfalfa cultivars compared to potato leafhopper-susceptible ones (Hansen et al., 2002). In their experiments, in the presence of large potato leafhopper populations, annual total forage yield of potato leafhopper-tolerant cultivars in plots not treated with insecticide was lower than that of potato leafhopper-susceptible ones in insecticide-treated plots in the seeding year and post-seeding years, by 0.29 Mg ha^{-1} and 0.95 Mg ha^{-1}, respectively. In the experiment of Sulc et al. (2015), forage yield at the first harvest in the first post-seeding year of a potato leafhopper-tolerant cultivar was 0.33 Mg ha^{-1} more than that of a potato leafhopper-susceptible cultivar, but 0.47 Mg ha^{-1} lower at the first harvest in the second post-seeding year, regardless of insecticide applications. The forage yield of alfalfa cultivars tolerant to potato leafhoppers and their tolerance levels are variable and might vary according to insect populations levels as well as environmental factors, such as drought and fall dormancy, among others (Lamp et al., 2007). Obermeyer (2020) reported that the insecticide treatment threshold for potato leafhopper-tolerant alfalfa was up to 10 times higher than that for susceptible cultivars. It is important to note that in some cases, according to some reports from the USA, in the absence of high potato leafhopper populations, yields of some potato leafhopper-tolerant alfalfa cultivars could be up to 15% lower than those of susceptible cultivars. (Hansen et al., 2002; Chasen et al., 2013; Wiersma and Thomas, 2016). According to seed companies, newer alfalfa cultivars offer superior performance with or without potato leafhoppers. Therefore, it is necessary to evaluate the performance of different alfalfa cultivars in Quebec, including with varying potato leafhopper populations, and to assess whether these cultivars can tolerate high potato leafhopper populations and if yes what is their yield advantage compared to susceptible cultivars. Given that potato leafhopper populations vary significantly from year to year it is also important to determine if tolerant cultivars are as performant as susceptible ones when potato leafhopper populations are low.

2.3.3. Alfalfa-grass mixtures

Mixing alfalfa with other species that are non-host of potato leafhoppers is another method that has been suggested to reduce the effect of this insect on alfalfa and which could contribute to reducing insecticide use. Growing alfalfa in mixtures with grasses is one strategy that can change microclimatic conditions, which in turn might reduce the attractiveness of the field to potato leafhoppers (Roda et al., 1997). Growing alfalfa mixed with grasses may help reduce damage caused by potato leafhoppers to alfalfa, which in turn increase forage yields and improve the forage nutritive quality (Bélanger et al., 2014). Studies in the United States have shown that potato leafhopper populations in alfalfa fields with as little as 9% grasses [smooth bromegrass (*Bromus inermis* Leyss.) and orchardgrass (*Dactylis glomerata* L.)] were 4 to 37% lower than those in pure alfalfa fields (Roda et al., 1997). Furthermore, according to a more recent study, Straub et al. (2013) reported that growing alfalfa with grasses helps protect alfalfa from the damage of hopperburn possibly by reducing the PLH propagation frequency. Straub et al. (2014) found that although there were fewer PLH nymphs per stem on alfalfa plants in alfalfa-orchardgrass plots than that in pure alfalfa plots, the fresh weight of alfalfa stems was similar in both conditions, possibly because grass intercropping interferes with the ability of adult PLH to locate alfalfa and thus reduce the probability of laying eggs on alfalfa. However, Degooyer et al. (1999) reported that growing alfalfa, orchardgrass and smooth bromegrass (*Bromus inermis* Leyss.) significantly lowered potato leafhopper populations, but failed to keep them below the threshold. Additionally, Chasen et al. (2013) found that growing alfalfa and orchardgrass together exerted no significant effects on potato leafhopper populations. It seems that mixtures of grasses and alfalfa can contribute to reducing population and their effect on alfalfa, however the optimal of alfalfa-grass mixture proportion remains to be determined.

2.4. NUTRITIVE VALUE

The nutritional value of alfalfa plays a key role in profitable dairy production, with the concentration of neutral detergent fiber (NDF), acid detergent fiber (ADF) and crude protein (CP) being crucial (Wood et al., 2018). Forage nutritive value is closely tied to its fiber concentration, as it directly impacts feed intake and digestibility in dairy cows (Nikolova et al., 2018). The NDF, a predictor of intake potential, includes cellulose, hemicellulose, and lignin (Robinson, 1999). As a subset of NDF, ADF (including cellulose and lignin) is also an important indicator of the digestibility of the forage (Robinson, 1999). According to studies carried out in the United States, the presence of potato leafhopper can lead to decreases in CP concentration of 0.7 to 2.3% in alfalfa when compared to unaffected plants (Sulc et al., 2004; Sulc et al., 2015). Older studies have suggested that the presence of potato leafhoppers might lead to even greater decrease in CP concentration, up to 35% (Flinn et al., 1990). In the experiment of Hansen et al. (2002), CP concentration of potato leafhopper-tolerant cultivars was 9 g kg^{-1} higher than that of potato leafhopper-susceptible ones. Sulc et al. (2004) reported that the average CP concentration from all harvests of potato leafhopper-tolerant cultivars was 11 g $kg^{-1}$and 13 g kg^{-1} higher than that of potato leafhopper-susceptible ones in insecticide-treated and untreated plots, respectively. The NDF concentration of potato leafhopper-tolerant cultivars was 15 to 25 g kg^{-1} lower in insecticide-treated plots compared to potato leafhopper-susceptible cultivars (Sulc et al., 2004). However, the impact of potato leafhopper on NDF concentration and on forage digestibility appears to be variable but might reduce NDF content and increase digestibility, as plant growth can be greatly affected by their presence, plants affected being much shorter and thus having a larger proportion of leaves compared to stems than plants not affected by potato leafhoppers (Sulc et al., 2004; Sulc et al. 2015). However, this apparent improvement in nutritive value is offset by the significant yield loss associated with it.

CONNECTING TEXT FOR CHAPTER 3

In the previous chapter, we reviewed the importance of alfalfa and the effect of potato leafhoppers on forage production, and presented previous research conducted on different management strategies to control potato leafhoppers in alfalfa fields. The use of insecticides applied following specific insect population thresholds is currently the most effective approach. The effectiveness of potato leafhopper-tolerant cultivars vary depending on environmental conditions, insect pressures, and stand age. Another management strategy consists in using mixtures of alfalfa and grasses. Adding grasses to alfalfa fields has the potential to reduce the overall impact of potato leafhoppers on alfalfa but also on forage production as yield losses may be compensated by grass production.

In the following chapter, we investigated forage yield and nutritive value of alfalfa cultivars with contrasted tolerance to potato leafhoppers with or without insecticide treatments at two contrasted locations in Quebec. The primary goal of this experiment is to quantify forage yield losses and changes in nutritive value associated with the presence of this insect in alfalfa fields of Quebec and evaluate locally the performance of several glandular-haired tolerant alfalfa cultivars comparing it to that of susceptible cultivars. Section 3.2 (Materials and methods) was initially developed by Philippe Seguin (McGill), Céline Georlette (CDBQ), Huguette Martel (MAPAQ), and Julien Saguez (CEROM), with input from Annie Claessens (AAFC). Field work presented in Section 3.2.2, was done in most parts by Xiawei Shi and supervised by P. Seguin at Sainte-Anne-de-Bellevue and by J. Saguez and his team at La Pocatière. Laboratory work and statistical analyses presented in Section 3.2.3 and 3.2.4, were done by X. Shi and supervised by P. Seguin. The results, discussions, and conclusions sections were primarily written by X. Shi and revised by P. Seguin.

CHAPTER 3

Forage yield and nutritive value of potato leafhopper-tolerant and susceptible alfalfa cultivars grown in Quebec

3.1. INTRODUCTION

The dairy industry plays a crucial role in Canadian agriculture, accounting for 1.8 million dairy cows. Over 70% of dairy cows are in Quebec and Ontario, contributing to a forage-based livestock industry (Statistics Canada, 2017; Statistics Canada, 2023). Alfalfa (*Medicago sativa* L.) one of the main perennial forage crops, is the most widely used species, ranking as the fourth largest crop in land area seeded in Canada, with more than 3 million hectares of pure alfalfa and alfalfa mixtures (Boucher et al., 2023; Statistics Canada, 2023). Regarded as the queen of forages, alfalfa is known for its high yield and nutritive richness, containing a significant amount of protein, along with various vitamins and minerals (Kumar, 2011; Liang et al., 2019).

Forage production is challenged by climate change which increases the frequency and intensity of abiotic and biotic stresses (Singer et al., 2018; Yan et al., 2023). These stresses reduce forage yields, pushing some producers to buy hay at sometimes high prices, thus affecting farm profitability. The potato leafhopper [PLH; *Empoasca fabae* (Harris); Homoptera: Cicadellidae], native to North America, is a significant agricultural pest of alfalfa in the eastern United States and some parts of Canada (Sulc et al., 2001; Chasen et al., 2014). Recently, the potato leafhopper has become a recurring problem in several regions of Quebec, contributing to reductions in forage production. The presence of potato leafhoppers in alfalfa fields is associated with reductions of 13 to 27% in forage yield, 3 to 39% in crude protein (CP), and significant declines in the concentrations of various nutrients, including vitamin A, carotene, calcium, phosphorus, and digestible dry matter (Nielsen et al., 1990; Sulc et al., 2004). As an economic pest, alfalfa yield losses due to potato leafhopper damage in the north-central and northeastern United States were valued to be as high as $66/ha several years ago (Lamp et al., 1991). More recently, potato leafhopper feeding has been estimated to cause alfalfa yield representing annual losses of approximately $500 per hectares in some parts of Ontario (Quesnel, 2012).

Potato leafhoppers feed by inserting their stylets into alfalfa tissues, repeatedly penetrating the vascular tissues and delivering saliva as they ruptures and ingests nutrients (Chasen et al., 2014). The feeding process triggers plant wound responses, including reduction of photosynthetic rate, stomatal conductance, and internode elongation, which in turn result in forage yield reduction and changes in the concentration of protein and fiber in alfalfa. The characteristic symptom of the attack of potato leafhoppers in alfalfa is known as “hopperburn” on the leaflets, starting as a yellow "V" shape and spreading from the center to the tip (Lamp et al., 2007; Chasen et al., 2014; Sulc et al., 2015).

Potato leafhoppers inflict particularly severe damage on establishing alfalfa stands, sometimes to the extent that it may result in plant death and reduced stand density (Davis and Fick, 1995). In post-establishment years, potato leafhoppers usually do not affect the first harvest in the Northeast USA and Ontario, since it migrates to the region between mid-May and late June, based on the spring climate conditions in the southern

USA, as it does not overwinter in the Northeast USA and Canada. However, during the growing season, potato leafhopper populations can significantly increase due to their rapid reproduction in the Northeast (Davis and Fick, 1995; Sulc et al., 2001; Chasen et al., 2014). Weekly field monitoring during summer is essential because potato leafhopper feeding leads to a decline in forage yield and quality even before severe leaf yellowing and "hopperburn" is observed (Hansen et al., 2002).

Historically, scouting and insecticide applications have been the primarily effective strategies used for controlling potato leafhoppers in alfalfa (DeGooyer et al., 1998; Chasen et al., 2014). According to Hammond et al. (2014), timely insecticide applications combined with weekly scouting played a crucial role in mitigating potato leafhopper activity, which in turn, contributed to improvement of alfalfa yield. Faris et al. (1981) also reported that potato leafhopper feedings reduced average total forage yield by 17% in the seeding year and post-seeding year compared to when treated with insecticides. Insecticide applications increased alfalfa CP concentration by 6% compared to when not treated. However, in 1997, some glandular-haired tolerant alfalfa cultivars became commercially available (Elden and McCaslin, 1997). The tolerance to potato leafhoppers is attributed to the presence of glandular hairs on the surface of leaves, stems, and other plant tissues, with chemical and physical resistance mechanisms for antibiosis and antixenosis (Shockley et al., 2002). These glandular hairs can ooze tiny droplets of sticky materials to reduce the feeding rate and egg production of the potato leafhopper, blocking the movement of potato leafhopper adults and nymphs (Sulc et al., 2014; Obermeyer 2020). The release of these cultivars has been recognized as a significant advancement in enhancing tolerance to potato leafhopper that allowed for a reduction in insecticide applications (Sulc et al., 2001). However, the performance of the glandular-haired cultivars in terms of yield, tolerance, and the nutritive value has been reported to vary according to environmental conditions and potato leafhopper population levels (Sulc et al., 2015). For example, yield gains of 50% for potato leafhopper-tolerant cultivars compared to susceptible ones have been reported by Sulc et al. (2001) in the presence of high potato leafhopper populations in a study carried out in the Midwest US. Gains in yields were, however, observed only one year out of four in a trial in New York state (Hansen et al., 2002).

Although research conducted in the USA has well documented the effect of potato leafhopper feeding on alfalfa production and evaluated the performance of tolerant cultivars in several regions, there is currently limited information available in Quebec. Our objective was to quantify forage yield losses and changes in nutritive value associated with the presence of this insect in Quebec alfalfa fields and locally evaluate the performance of glandular-haired tolerant alfalfa cultivars compared to susceptible cultivars, which to our knowledge has never been reported.

3.2. MATERIALS AND METHODS

3.2.1. Sites and treatments description

An experiment was conducted at two contrasting sites in Quebec: Sainte-Anne-de-Bellevue (SAB, 2332 cumulated growing degree days on a 5 °C basis [GDD5]), on a Chicot sandy loam (45° 25' 38.0" N lat., 73° 55'45.0" W long.) and La Pocatière (POC, 1846 cumulated GDD5), on a Kamouraska heavy clay soil (47° 21'21.0" N lat., 70° 1' 55.0" W long.).

At each of the two sites, seven alfalfa cultivars were evaluated, including five potato leafhopper-tolerant cultivars (FSG421LH, Safeguard PLH, SW315LH, WL358LH and 55H94) and two locally adapted susceptible cultivars (Eclipse and Dominator). Alfalfa was seeded at a rate of 15 kg ha^{-1} on a pure-live seed basis at a targeted depth of 10 mm and rows spaced at 18 cm using a Fabro seven-row seeder (Swift Current, SK, Canada) at SAB and a Kincaid seven-row seeder (Haven, KS) at POC (CRAAQ, 2005). Plots were seeded in the spring of 2021 at both sites and were harvested in 2021, 2022 and 2023. Plots sizes varied depending on the site, but were of a minimum area of 1.3 × 5 m. Each cultivar was also subjected to two insecticide treatments, with and without. Each treatment was replicated four times resulting in a total of 56 plots at each site.

Plots were managed following local recommendations with fertilization being done before seeding based on soil analyses and weed control using herbicides in the seeding year to reduce weed pressure (CRAAQ, 2010; OMAFRA, 2021). Plots were treated with insecticides (Matador 120EC) when potato leafhopper populations reached locally recommended threshold for treatment, the intervention thresholds are evolutive and depend on alfalfa height (Table 3.1) (RAP, 2020).

Matador 120EC [Syngenta Crop Protection Canada, Guelph, ON, Canada; with lambda-cyhalothrin (120 g L^{-1})] was applied at a rate of 83 mL ha each time the potato leafhopper population exceeded the treatment threshold. This resulted in three applications in the seeding year at SAB (i.e., June 9, June 23, and July 23) and one application at POC (i.e., July 27), and none in the two post-seeding years at both sites. All plots were harvested one or two times in the seeding year and three to four times in post-seeding years, the number of harvests depended on the location and environmental conditions.

The experimental design was a randomized complete block with split-plot restriction and four replicates. Insecticide treatments were assigned to main plots and alfalfa cultivars to subplots. Buffer borders 4.5 m wide were seeded with a potato leafhopper-susceptible alfalfa cultivar around each main plot as per Sulc et al. (2014).

3.2.2. Data collection

Plot harvests were done based on a 450-500 GDD5 interval between each harvest or when plants reached the early flowering stage. A 0.6 × 5 m strip was harvested in each plot with a forage small-plot flail forage harvester to determine forage yields. A 500 g fresh sample was collected and dried at 55 °C for at least 48 hours in a forced-air oven to determine forage yields on a dry matter basis. The dried samples were then grinded through a 1 mm-sieve using a Willey mill (Standard model 4, Arthur H. Thomas Co., Philadelphia, PA). These ground samples were later used to determine the nutritional value of the harvested forage. At each harvest, alfalfa height was also measured using ten randomly selected plants in each plot in order to assess the effects caused by potato leafhoppers on plant development. The vegetation from a permanent quadrat of 0.5 × 0.5 m^2 located in each plot was also harvested using scissors and collected samples were separated to alfalfa and weeds by hand, with each component being dried at 55 °C for at least 48 hours to assess yield contribution on a dry matter basis. No weeds were observed in the post-seeding years at both sites. Only alfalfa yields are reported herein, weeds biomass being subtracted from the total biomass harvested.

Potato leafhopper populations (i.e., sum of adults and nymphs) were monitored weekly at each site in each of the main plots from June to August, using an established protocol (RAP, 2020). Briefly, 10 sweeps were performed using a net with a 30-40 cm diameter avoiding the first meter on each side of the main plots. Insects collected were frozen for at least 1 hour and then the total number of potato leafhopper collected (i.e., sum of adults and nymphs) were determined.

3.2.3. Nutritive value analyses

The forage nutritive value of ground samples from each plot at all harvests was assessed by determining crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) concentrations. The ANKOM filter bag technique using the ANKOM200 Fiber Analyzer (ANKOM Technology, Macedon, NY) was used to determine NDF and ADF concentrations. The method of Mertens (2002), which uses heat-stabilized a-amylase and sodium sulfite was used for NDF concentration determination. The ADF concentration was determined following the method of the Association of Official Analytical Chemists (AOAC, 1990). Finally, the CP concentration was determined from the total nitrogen (TN) with the equation CP = TN × 6.25. The procedure of Simili da Silva et al. (2014) was used to determine TN. After the samples were mineralized in a mixture of sulfuric and selenious acids, the N concentration was determined using an automatic analyzer (QuikChem 8000 Lachat Zellweger Analytics Inc., Lachat

Instruments, Milwaukee, WI) following the method 13-107-06-2-E (Lachat Instruments, 2011). The annual nutritive values presented are annual weighted averages based on the individual contribution of each harvest to total annual forage yield.

3.2.4. Statistical analyses

Data were analyzed with a two-way analysis of variance (ANOVA) using PROC GLM of the SAS software (SAS Institute, 2014). Replicates for each site were considered a random effect while insecticide and cultivar effects were considered fixed. When cultivar × insecticide interactions were significant, data were further analyzed using one-way ANOVA. Differences between treatments means were determined using the Least Significant Difference (LSD) test at the significance level of $\alpha = 0.05$. Only significant effects ($P < 0.05$) are discussed herein.

3.3 RESULTS

3.3.1. Potato leafhopper populations and insecticide applications

In the seeding year, there were three times at SAB and once at POC, when potato leafhopper populations exceeded the treatment threshold, Maximum populations observed during the season were 29 and 45 insects per ten sweeps at SAB and POC, respectively (Figure 3.1). Thus, using recommendations (Table 3.1), based on populations observed, two insecticide applications were applied (9 June and 23 June 2021) before the first harvest and another (23 July 2021) before the second harvest at SAB. At POC, only one insecticide application was done (27 July 2021) before the only harvest was made (Figure 3.1). Differences in potato leafhopper populations between insecticide treatments were observed at five dates at SAB (15 June, 5 July, 27 July, and 2 August 2021) and two dates at POC (2 August and 10 August 2021) (Figure 3.1).

Potato leafhopper populations were low at both sites in both post-seeding years (Figure 3.1). In the first post-seeding year, insect number ranged from 0 to 11 and 0 to 1.25 insects per ten sweeps at SAB and POC, respectively, never reaching the threshold level required for insecticide applications. In the second post-seeding year, potato leafhopper populations were again low at both sites, ranging from 0 to 5 and 0 to 1 insect per ten sweeps at SAB and POC, respectively, again never reaching the threshold levels required for insecticide applications.

3.3.2. Plant height and alfalfa yield in the seeding year

In the seeding year, there were two forage harvests in SAB and one in POC. At SAB, differences in alfalfa yield between insecticide treatments or cultivars were minimal in the seeding year, no treatment main effects or interactions being observed ($P > 0.05$). However, insecticide application resulted in 32% greater average alfalfa plant height at the first harvest (Data not shown, $P < 0.05$; see Figure A1) when compared to plots not treated with insecticide. In addition, differences were observed at the second harvest (Data not shown, $P < 0.05$; see Figure A2), among the seven alfalfa cultivars evaluated, 55H94 (a potato leafhopper-tolerant cultivar) being 19% taller than the other cultivars, except for another potato leafhopper-tolerant cultivar (FSG421LH).

At POC, despite high potato leafhopper populations for part of the season, the application of insecticide did not affect alfalfa yield nor plant height at harvest ($P > 0.05$). Differences between alfalfa cultivars were observed, no interaction between insecticides and cultivars, however, being observed ($P > 0.05$). Alfalfa plant height at harvest of SW315LH was 17% lower than that of other cultivars, except for Eclipse (a potato leafhopper-susceptible cultivar) and WL358LH (a potato leafhopper-tolerant cultivar) (Data not shown, $P < 0.001$; see Figure A3).

3.3.3. Plant height and alfalfa yield in post-seeding years

In the first post-seeding year, there were four forage harvests at SAB and three at POC. At SAB, we observed an insecticide main effect for alfalfa yield at the first harvest ($P < 0.01$), a cultivar main effect for alfalfa yield at the first, second, and third harvests as well as the annual yield, however, an insecticide × cultivar interaction for annual alfalfa yield was also observed ($P < 0.05$). Finally, a cultivar main effect was observed for alfalfa height at the second, third and fourth harvests ($P < 0.01$). At POC, a cultivar main effect was observed for alfalfa yield at the third harvest and for the annual yield, as well as for alfalfa height at the second and third harvests ($P < 0.05$).

At SAB, it is important to note that the insecticide response at the first harvest reflected a residual response to the insecticide treatments done in the previous year as no insecticide applications were done in post-seeding years. Alfalfa yield at the first harvest was 7% higher in plots submitted to three insecticide applications in the seeding year (Figure 3.2; $P < 0.01$), when compared to plots that were untreated. The significant insecticide × cultivar interaction for the annual alfalfa yield (Figure 3.3; $P < 0.05$) reflected that cultivars did not respond similarly to insecticide applications done in the previous year. The only cultivars that showed a positive residual response to insecticide applications done in the previous year were two purportedly potato leafhopper-tolerant cultivars (i.e., 55H94 and Safeguard PLH), for which alfalfa yield was 12% greater if treated compared to if not treated in the previous year with insecticides. None of the other cultivars responded to insecticides. The cultivar main effects observed for the first, second, third harvests and the annual yield reflected that overall two of the tolerant cultivars (WL358LH and SW315LH) were lower yielding compared to the other five cultivars (Data not shown, $P < 0.05$; See Figures A4-A7). These two cultivars were consistently shorter than all other cultivars, 22% at the second harvest, 12% at the third harvest, and 18% at the fourth harvest (Data not shown, $P < 0.01$; see Figures A8-A10). Similarly, at POC, WL358LH and SW315LH were 10% shorter than potato leafhopper-susceptible cultivars at the second harvest, and both were 16% shorter than all other cultivars at the third harvest (Data not shown, $P < 0.01$; see Figures A11-A12). Differences between cultivars at POC were inconsistent and overall minimal with maximum differences between cultivars being of 9% (Data not shown, $P < 0.05$; see Figures A13-A14)

In the second post-seeding year, there were three forage harvests at SAB and two at POC. Overall, only cultivar main effects were observed for alfalfa yields and heights at both sites. Differences in alfalfa yields were observed at the third harvest in SAB ($P < 0.05$) and at both harvests and annual yield in POC ($P < 0.01$). Finally, differences in alfalfa heights were observed at each harvest at both sites ($P < 0.05$).

At SAB, the cultivars FSG421LH, 55H94 and Safeguard PLH (potato leafhopper-tolerant cultivars) yielded 14% more than the other cultivars at the third harvest, except for Eclipse, one of the two potato leafhopper-susceptible cultivars (Data not shown, P

< 0.05; see Figure A15). At this site, differences were also observed between cultivars in average plants height at harvest. Cultivar SW315LH a potato leafhopper-tolerant cultivar was shorter than all other cultivars at each harvest, being 7, 11, and 16% shorter at the first, second and third harvest, respectively (Data not shown, P < 0.01; see Figures A16-A18).

At POC, the annual alfalfa yield of SW315LH and 55H94 (potato leafhopper-tolerant cultivars) was 27% higher than that of all other cultivars regardless of the insecticide treatment made in the seeding year (Data not shown, P < 0.01; see Figure A19), a similar differences being observed at both harvests. 55H94 was the highest yielding at both harvests, joined by SW315LH at the first harvest (Data not shown, P < 0.05; see Figures A20-A21) The high yield observed for SW315LH is contrasting with its yields observed in the seeding year which was significantly lower than that of all other cultivars. Finally, differences in average plant height were also observed at the first and second harvests. At the first harvest, height was the highest for Dominator and lowest for SW315LH, while at the second harvest, WL358LH was 12% shorter than all other cultivars except for Eclipse (Data not shown, P < 0.01; see Figures A22-23). Overall, there was no correlation between plant height and alfalfa yields at POC.

3.3.4. Forage nutritive value in the seeding year

In the seeding year, treatment response varied depending on the site. At SAB, an insecticide × cultivar interaction was observed for CP concentration (P < 0.01) and an insecticide main effect for ADF concentration (P < 0.05). While at POC, both insecticide and cultivar main effects for CP concentration (P < 0.05) and a cultivar main effect for ADF concentration were observed (P < 0.01).

The insecticide × cultivar interaction observed at SAB for CP concentration reflected a difference in cultivar response to insecticide treatments (Figure 3.4; P < 0.01). For potato leafhopper-susceptible cultivars, the three insecticide applications increased the average CP concentration by 17%, compared to when no insecticide were applied with high potato leafhopper populations. In contrast, for potato leafhopper-tolerant cultivars, insecticide treatments increased the average CP concentration for only two out of five cultivars, with 7 and 13% increases for Safeguard PLH and Wl358LH, respectively. When not treated with insecticides, the average CP concentration of two potato leafhopper-tolerant cultivars (55H94 and SW315LH) was 9% higher than that of potato leafhopper-susceptible cultivars (Figure 3.4). The insecticide treatment main effect observed at SAB reflected that across all cultivars, insecticide applications increased ADF concentration by 7% compared to when insecticides were not applied (Data not shown, P < 0.05; see Figure A24).

At POC, one insecticide application increased the CP concentration of the seven cultivars by an average of 6% (Data not shown, P < 0.05; see Figure A25). Among the seven alfalfa cultivars evaluated, the CP concentration of Eclipse (a potato leafhopper-susceptible cultivar) was 7% lower than that of the other potato leafhopper-susceptible cultivar (Dominator) and two potato leafhopper-tolerant cultivars (FSG421LH and 55H94) regardless of insecticide treatment (Table 3.3; P < 0.05). Differences between cultivars were also observed for the ADF concentration, that of Eclipse being 9% lower than that of the remaining six cultivars (Table 3.3; $P < 0.01$).

3.3.5. Forage nutritive value in post-seeding years

In the first post-seeding year with low potato leafhopper populations, no effects of insecticide treatments done in the previous year or insecticide × cultivar interactions were observed for any of the forage nutritive value attributes at SAB ($P > 0.05$). However, an insecticide × cultivar interaction ($P < 0.01$) was observed at POC for CP concentration. Differences between cultivars were although observed at both sites for most forage quality attributes ($P < 0.05$). At SAB, CP concentration of SW315LH (a potato leafhopper-tolerant cultivar) was 7% higher than that of the remaining five alfalfa cultivars except for WL358LH (another potato leafhopper-tolerant cultivar) (Table 3.3; $P < 0.01$). The reverse was observed for ADF concentration as it was 6% lower for SW315LH than all other cultivars, except for WL358LH (Table 3.3; $P < 0.01$). The NDF concentration of FSG421LH and 55H94 (two potato leafhopper-tolerant cultivars) was 5% higher than all other cultivars, except for Safeguard PLH (a potato leafhopper-tolerant cultivar) (Table 3.3; $P < 0.01$).

At POC, a significant insecticide × cultivar interaction was observed for CP concentration ($P < 0.01$). Following treatment with insecticide in the seeding year, the CP concentration of WL358LH (a potato leafhopper-tolerant cultivar) was 11% higher than that of two potato leafhopper-susceptible cultivars and two other potato leafhopper-tolerant cultivars (i.e., 55H94 and SW315LH). Insecticide application made in the seeding year with high potato leafhopper populations also increased CP concentration of FSG421LH, 55H94 and WL358LH (three potato leafhopper-tolerant cultivars), by 9, 8 and 8%, respectively compared to when no insecticide was used. The CP concentration of the other cultivars was not affected by the insecticide application made in the previous year (Figure 3.5). In terms of fiber concentrations at POC, the ADF and NDF concentrations of 55H94 (a potato leafhopper-tolerant cultivar) were 7 and 6% higher, respectively, compared to the other five alfalfa cultivars, except for one potato leafhopper-susceptible cultivar (i.e., Dominator) (Table 3.3; $P < 0.01$). The ADF and NDF concentrations of WL358LH (a potato leafhopper-tolerant cultivar) were 7 and 6% lower, respectively, compared to the other five alfalfa cultivars, except for one potato leafhopper-tolerant cultivar (Safeguard PLH) (Table 3.3).

In the second post-seeding year, there was even fewer treatment effects on nutritive value attributes. The only differences between cultivars were observed for CP, NDF and ADF concentrations at SAB, and for ADF concentration at POC. At SAB, the CP concentration of SW315LH (a PLH-tolerant cultivar) was 14% higher than that of the other cultivars (Table 3.3; $P < 0.01$). By contrast, the average ADF and NDF concentrations of SW315LH were 10 and 9% lower, respectively, than those of the remaining cultivars at SAB (Table 3.3; $P < 0.01$). An insecticide × cultivar interaction was also significant for NDF concentration, however, it only reflected a difference in magnitude of cultivars ranking between plots treated with or without insecticides, concentration being the lowest with SW315LH in both situations (Data not shown, $P < 0.05$; see Figure A26). Similarly to what was observed in the first post-seeding year, SW315LH had the shortest plants, highest CP concentrations and lowest NDF and ADF concentrations in the second post-seeding year. Finally, at POC, as observed at SAB, ADF concentration was the lowest for SW315LH (and Dominator), no differences with 55H94 and WL358LH, however, being observed (Table 3.3; $P < 0.05$).

3.4. DISCUSSION

Large potato leafhopper populations that required insecticide treatments were observed in the seeding year at both sites. In contrast, populations remained low in post-seeding years, populations always being below thresholds for insecticide treatments (Figure 3.1). In recent years, potato leafhopper populations have been highly variable in Quebec between years and regions. For example, according to local scouting reports, very large populations were observed in several regions in 2020, compared to only few regions in 2019, and overall very rare reports in 2018 (CRAAQ, 2023). Taylor and Shields (2018) reported that the population of potato leafhopper depends on several factors including temperature, rainfall, and host plants present. Being a migratory insect, it is therefore difficult to predict its occurrence beforehand.

Each insecticide application was made when potato leafhopper thresholds were reached, significantly reducing potato leafhopper populations (Figure 3.2). Hammond et al. (2014) reported that timely insecticide applications along with weekly scouting mitigated potato leafhopper activity, consequently contributing to improved alfalfa yield. While insecticides can effectively reduce potato leafhopper populations in alfalfa fields, within 2 to 3 weeks insects may return to the threshold levels in optimal environmental conditions (Sulc et al., 2014).

Each harvest in our experiment, in either seeding year or post-seeding years, also directly reduced potato leafhopper populations (Figure 3.2). Hammond et al. (2014) suggested that with high potato leafhopper populations a four-harvest system might be preferable to a three-harvest one as timely harvests can impede the multiplication of potato leafhopper generations, while delayed harvests facilitate the transition of nymphs to adults. Similarly, Undersander et al. (2011) reported that an early harvest was preferable over insecticide applications, if insect populations exceeded the threshold within 7 days of a scheduled harvest. The earlier harvest serves as a preventive approach against additional potato leafhopper feedings, since eggs and nymphs, with limited mobility, are exposed to a hot and dry environment, ultimately resulting in their mortality (Chasen et al., 2014).

In the seeding year at SAB, the limited difference in alfalfa yields we observed between potato leafhopper-tolerant and susceptible-cultivars despite the presence of high potato leafhopper populations (Table 3.2), is in agreement with Hansen et al. (2002). They indeed also failed to observe significant differences in central New York in forage yield at both of two harvests in the seeding year between potato leafhopper-tolerant and susceptible alfalfa cultivars with potato leafhopper populations reaching the threshold for treatment after the first harvest. In contrast in Western New York (Hansen et al., 2002), where insect populations reached the treatment threshold before the first harvest, potato leafhopper-tolerant cultivars yielded 0.5 Mg ha^{-1} more than that susceptible cultivars at the first harvest. Benefits of tolerant cultivars might be associated with the time at which potato leafhopper arrive in a specific region, which rarely happens before early to mid-June in most regions of Quebec. Our data suggest that tolerant cultivars may be of limited benefits in Quebec to reduce the effects on alfalfa yields of high populations of potato leafhoppers in the seeding year.

In the seeding year at both sites, difference in annual alfalfa yields between insecticide treatments were minimal, which is in agreement with results reported from Minnesota and Wisconsin by Sulc et al. (2001). In contrast, in Indiana, they reported that while there was no difference between insecticide treatments for potato leafhopper-tolerant cultivars, insecticide treatments increased the annual total forage yield of susceptible cultivars, compared to when not treated. In Ohio, difference between insecticide treatments was significant for both potato leafhopper-tolerant and susceptible cultivars. They thus concluded that the response of forage yield to insecticide treatments was variable, according to location, year, and cultivars.

In the seeding year in the presence of high potato leafhopper populations, insecticide treatments overall positively affect alfalfa CP concentration at both sites. At POC, insecticide treatment increased CP concentration of all cultivars by 6% compared to when not treated (Data not shown; see Figure A25), while at SAB, CP concentrations of potato leafhopper-susceptible cultivars and two of five tolerant cultivars were overall increased by 13% by insecticide applications (Figure 3.4). The positive effect on alfalfa CP concentration of insecticides applied in the seeding year when potato leafhopper populations are high has previously been well documented. For example, Faris et al. (1981) observed that alfalfa CP concentration was 6% higher when treated with insecticides than when not treated. Similarly, Sulc et al. (2015), reported that potato leafhopper-susceptible cultivars consistently exhibited higher CP concentration when treated with insecticides.

At SAB, the use of some potato leafhopper-tolerant cultivars reduced the impact of insects on CP concentrations when no insecticides were applied. Indeed, in untreated plots, two of five potato leafhopper-tolerant cultivars had higher CP concentrations than susceptible ones, while there was no difference in insecticide-treated plots (Figure 3.4). Benefits of tolerant cultivars were also reported by Hansen et al. (2002). Indeed, they reported CP concentrations that were 21 g kg^{-1} higher than that of potato leafhopper-susceptible cultivars when facing high potato leafhopper populations but left untreated with insecticides. A similar response was also reported by McCaslin (1998). Such results demonstrate that the use of potato leafhopper-tolerant cultivars can reduce in some cases the negative effect of potato leafhoppers have on alfalfa CP concentration in the seeding year.

The presence of high potato leafhopper populations also had an effect on ADF concentration in the seeding year, their effect being the opposite of CP concentrations. At SAB, insecticide applications resulted in an increase in ADF concentration (Data not shown; see Figure A24), which is probably an indirect effect of insecticides on alfalfa plant height. Usually ADF concentration is higher in taller plants due to an increase in structural carbohydrates and lignin concentrations, which are quantified in the ADF procedure (Robinson, 1999). Similar effects of insecticides on ADF concentration were also reported in previous studies including Faris et al. (1981) and Hansen et al. (2002).

In the first post-seeding year, at SAB, insecticides applied in the previous year exerted a residual effect on alfalfa yield at the first harvest (Figure 3.2), although no residual effect were observed at POC. Similarly, Faris et al. (1981) reported that not making insecticides applications in the seeding year resulted in a 12% reduction of alfalfa yield in the first post-seeding year compared to when treated with insecticides. High populations of potato leafhopper and damage from their feeding can result in alfalfa yield reductions in subsequent harvests or years, as it disrupts the transfer of photosynthetic products to the root and crown tissues of alfalfa (Lamp et al., 2001; Chasen et al., 2014).

In the first post-seeding year, at SAB, CP and ADF concentrations of SW315LH (a potato leafhopper-tolerant cultivar) were the highest and lowest, respectively, compared to that of other cultivars evaluated, except for another potato leafhopper-tolerant cultivar (i.e., WL358LH) (Table 3.3). These concentrations were correlated with alfalfa height, SW315LH being the shortest cultivar at the second, third and fourth harvest (except for WL358LH) (Data not shown; see Figures A8-10). There was a similar trend in the second post-seeding year at SAB, SW315LH had the highest CP and lowest ADF and NDF concentrations among the seven cultivars evaluated (Table 3.3), as SW315LH was the shortest at all three harvests (Data not shown; see Figures A16-18). As mentioned earlier, shorter plants usually have lower NDF and ADF concentrations and higher CP concentrations than taller plants (Andrzejewska et al., 2020). The response observed is thus probably not associated with the tolerance trait but rather to the different growth pattern of this cultivar compared to that of others.

Differences between cultivars either in terms of alfalfa yield or nutritive value varied among the different environments (sites and years), but in conditions where potato leafhopper populations were only high in the seeding year, the benefit of potato leafhopper-tolerant cultivars appeared to be limited. Sulc et al. (2001) also reported that there were no benefits or drawbacks according to the forage yield for using potato leafhopper-tolerant alfalfa cultivars when applying insecticides or under the lower potato leafhopper pressure. Some studies in the past have suggested that the use of tolerant cultivars in low potato leafhopper conditions might be associated to lower alfalfa yields compared to regular susceptible cultivars. According to some reports from the USA, when potato leafhopper populations were low, some potato leafhopper-tolerant alfalfa cultivars could yield up to 15% lower than susceptible cultivars. (Hansen et al., 2002; Chasen et al., 2013; Wiersma and Thomas, 2016), which was not observed in the present study.

3.5. CONCLUSIONS

Potato leafhopper populations vary from one year to another and between regions, but overall populations in the present study remained low in two out of three years, necessitating control only in the seeding year. In such conditions, insecticide applications, based on threshold defined by scouting, and harvesting were effective ways to reduce potato leafhopper populations. The use of insecticides, however, failed to significantly affect alfalfa forage yields in the seeding year, but had a small residual effect in the first post-seeding year at one of two sites. The use of insecticides, positively affected forage nutritive value in the seeding year of potato leafhopper-susceptible cultivars and some tolerant cultivars by increasing CP concentration at both sites. The benefit of tolerant cultivars appeared to be overall limited, their performance being comparable to that of susceptible cultivars in most conditions we experienced. The performance of potato leafhopper-tolerant cultivars and that of insecticides will need to be confirmed in a greater number of environments of Quebec before definite conclusions can be made regarding the benefit of increasing the use of tolerant cultivars.

Table 3.1. Insecticide intervention threshold according to the average number of potato leafhoppers and alfalfa plant height.

Plant height (cm)	Average number of potato leafhoppers per 10 sweeps
Under 15	4
15-19	6
20-24	8
25-29	10
30-34	12
35-39	14
40-44	16
45-49	18
50 and above	20

Adapted from RAP (2020).

Table 3.2. Annual alfalfa yield of five potato leafhopper-tolerant and two potato leafhopper-susceptible alfalfa cultivars in the seeding year (2021) and first two post-seeding years (2022 and 2023) at two contrasted sites in Quebec, Canada. Results are the average of two insecticide treatments (with and without) (n=8).

	Annual alfalfa yield					
	Sainte-Anne-de-Bellevue			La Pocatière		
Cultivar	2021	2022	2023	2021	2022	2023
	---------- kg DM ha^{-1} ----------					
Dominator‡	947	10 783a§	8790	1197	9064a	8774b
Eclipse	1218	10 466ab	8862	1295	8447b	7410cd
FSG421LH	1041	10 952a	9176	1444	8810ab	8477bc
55H94	1230	11 038a	9102	1249	9144a	10 013a
Safeguard PLH	1180	10 910a	9181	1311	9042a	7619bcd
WL358LH	958	9864bc	8868	1207	8320b	7226d
SW315LH	1000	9228c	8384	1105	8781ab	10 000a
SE†	114.6	229.8	214.0	88.9	181.7	426.9
	---------- P > F ----------					
	0.34	< 0.0001	0.14	0.22	0.02	< 0.0001

† SE, Standard Error (P = 0.05).
‡ Potato leafhopper-susceptible alfalfa cultivars = Dominator and Eclipse; Potato leafhopper-tolerant alfalfa cultivars = FSG421LH, 55H94, Safeguard PLH, WL358LH and SW315LH.
§ Within columns means for each cultivar followed by the same letter are not significantly different according to LSD (0.05).

Table 3.3. Forage crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) concentrations of five potato leafhopper-tolerant and two potato leafhopper-susceptible alfalfa cultivars in the seeding year (2021) and first two post-seeding years (2022 and 2023) at two contrasted sites in Quebec, Canada. Results are the average of two insecticide treatments (with and without) (n=8).

Cultivar	Sainte-Anne-de-Bellevue			La Pocatière		
	2021	2022	2023	2021	2022	2023
	CP					
	g kg^{-1}					
Dominator	214	223bc	180bc	187ab	203bcd	189
Eclipse	213	216c	176bc	177c	201cd	172
FSG421LH	212	218bc	173c	189ab	205bcd	178
55H94	218	216c	185b	192a	196d	178
Safeguard PLH	218	216c	176bc	183abc	212ab	174
WL358LH	215	228ab	181bc	182bc	217a	181
SW315LH	220	234a	203a	183bc	208abc	182
SE[†]	2.7	3.4	3.9	3.1	3.2	4.6
	P > F					
	0.32	0.003	< 0.0001	0.02	0.001	0.68
	NDF					
	g kg^{-1}					
Dominator	346	375bc	402bc	325	427a	470
Eclipse	334	379abc	401bc	304	409bc	498
FSG421LH	343	390a	416a	316	411b	487
55H94	345	390a	406abc	318	431a	476
Safeguard PLH	333	384ab	412ab	317	399cd	492
WL358LH	341	365c	395c	317	394d	486
SW315LH	341	367c	372d	315	414b	479
SE[†]	5.0	4.9	4.8	6.4	4.0	7.0
	P > F					
	0.41	0.002	< 0.0001	0.46	< 0.0001	0.11
	ADF					
	g kg^{-1}					
Dominator	218	296ab[§]	327ab	219a	348ab	368b
Eclipse	212	297ab	329ab	196b	334cd	394a
FSG421LH	217	301a	336a	216a	335cd	386a
55H94	224	305a	331ab	216a	353a	378ab
Safeguard PLH	222	302a	333ab	211a	326de	388a
WL358LH	220	287bc	323b	212a	320e	381ab
SW315LH	219	282c	299c	209a	339bc	368b
SE[†]	4.6	4.3	4.2	3.7	3.8	6.0
	P > F					
	0.65	0.005	< 0.0001	0.003	< 0.0001	0.03

[†] SE, Standard Error (P = 0.05).
[‡] S (potato leafhopper-susceptible alfalfa cultivars) = Dominator and Eclipse; T (potato leafhopper-tolerant alfalfa cultivars) = FSG421LH, 55H94, Safeguard PLH, WL358LH and SW315LH.
[§] Within columns means for each cultivar followed by the same letter are not significantly different according to LSD (0.05).

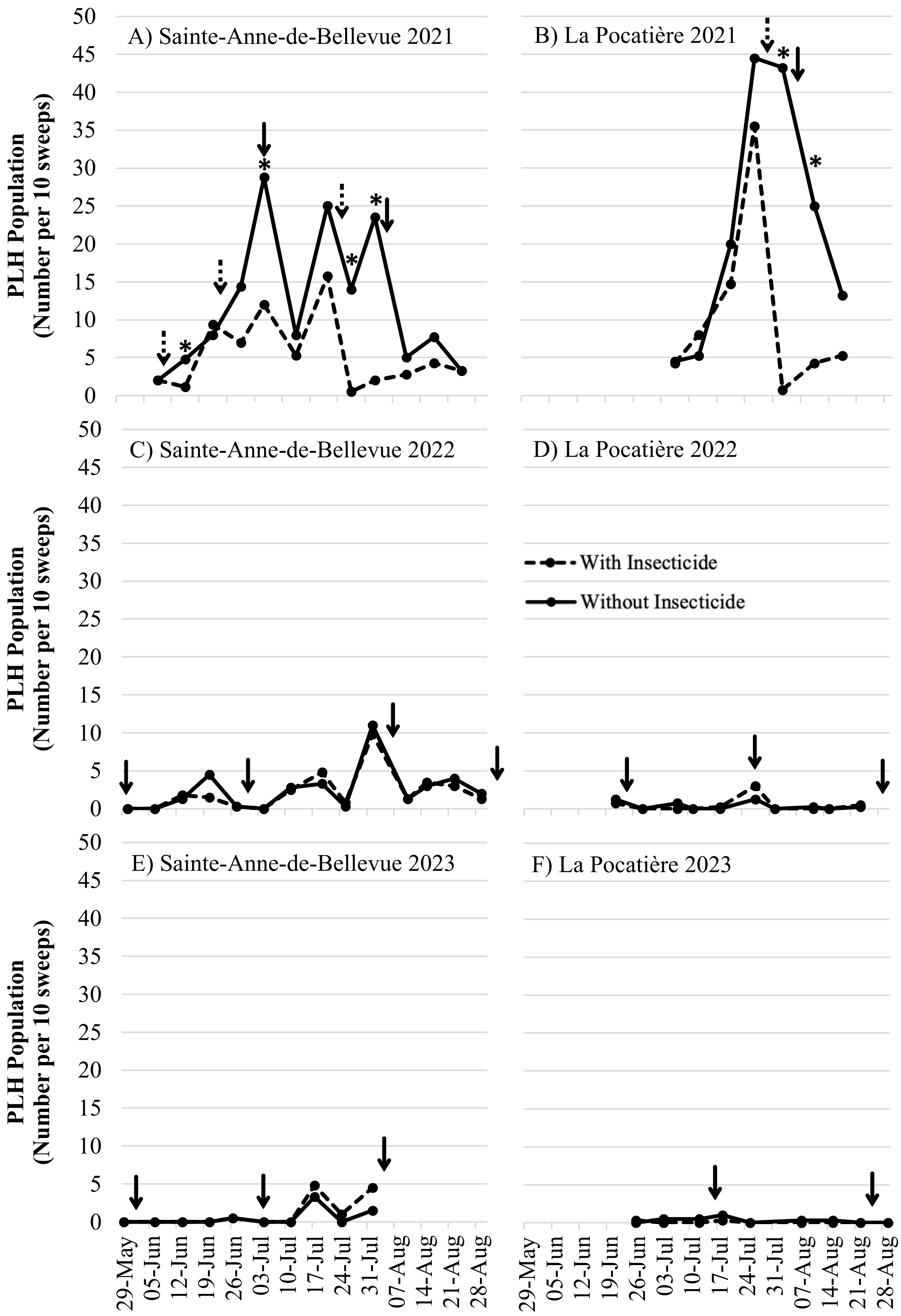

Figure 3.1. Potato leafhopper (PLH) populations during the seeding year 2021 and first two post-seeding years (2022 and 2023) at two contrasted sites in Quebec, Canada and submitted to two insecticide treatments (with and without)
*** = Differences between insecticide treatments (P < 0.05) at specific dates; ↓ = Forage harvests; ⇣ = Insecticide applications.**

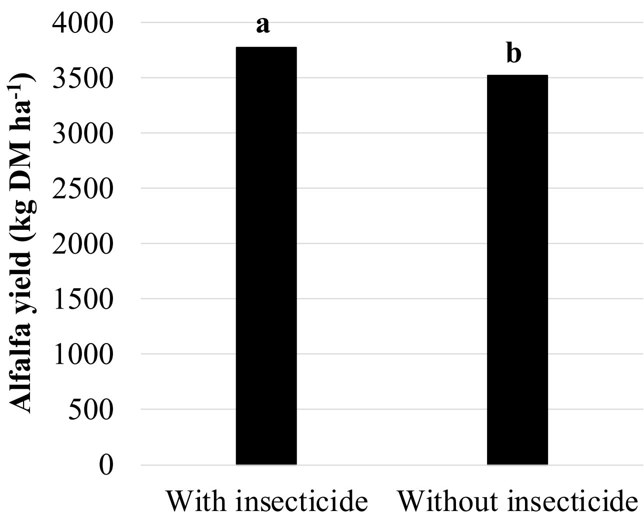

Figure 3.2. Alfalfa yield at the first harvest in the first post-seeding year at Sainte-Anne-de-Bellevue, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Insecticide applications were done in the seeding year. Values are the average of seven alfalfa cultivars.

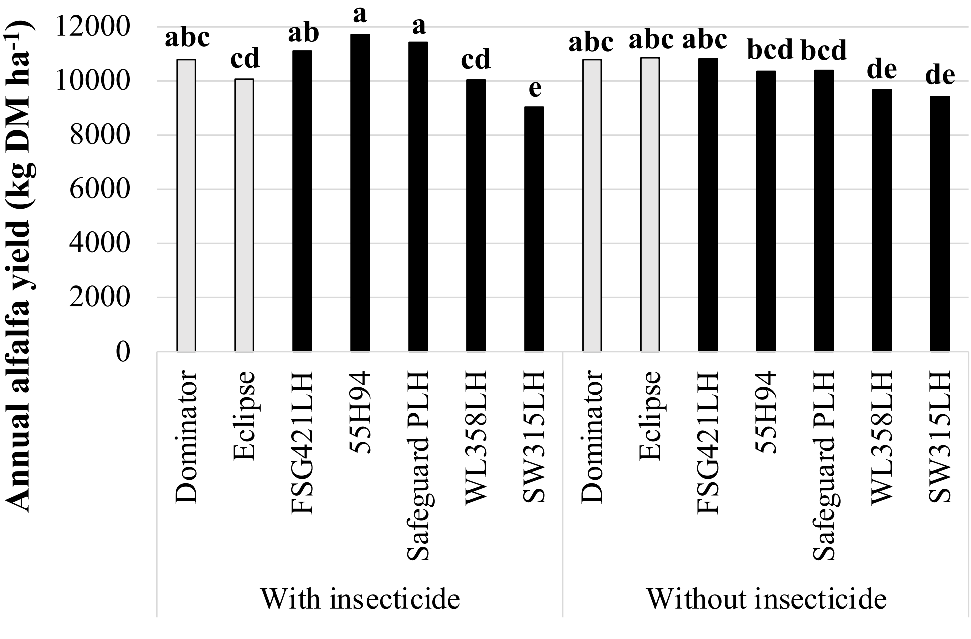

Figure 3.3. Annual alfalfa yield in the first post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at Sainte-Anne-de-Bellevue, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Insecticide applications were done in the seeding year.

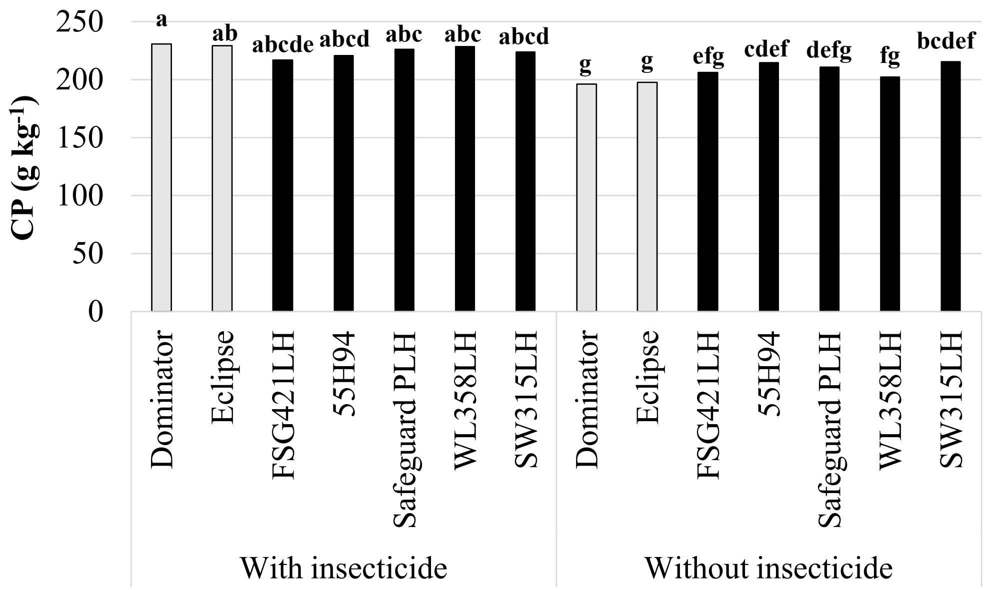

Figure 3.4. Average forage crude protein (CP) concentration in the seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at Sainte-Anne-de-Bellevue, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Three insecticide applications were done in the seeding year.

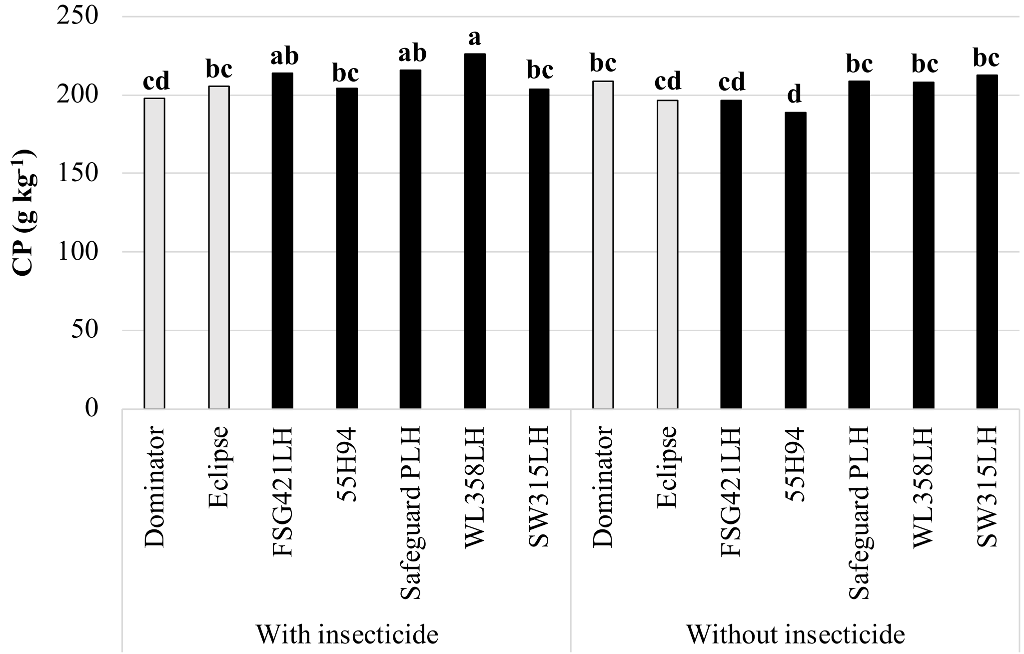

Figure 3.5. Average forage crude protein (CP) concentration in the first post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at La Pocatière, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Three insecticide applications were done in the seeding year.

CONNECTING TEXT FOR CHAPTER 4

In the previous chapter, forage yield and nutritive value of alfalfa cultivars with contrasted tolerance to potato leafhoppers with or without insecticide treatments were studied at two contrasted locations in Quebec. Results observed demonstrated that insecticides and timely harvesting could be effective ways to reduce potato leafhopper populations, while potato leafhopper-tolerant alfalfa cultivars performed variably according to environmental conditions and the level of the insect populations, but overall offered limited benefits in the conditions we experienced. If insecticides effectively reduced potato leafhopper populations, they failed to consistently affect alfalfa yields.

In the following chapter, the effect of an additional factor on the response of alfalfa to potato leafhoppers, that of alfalfa-grass mixtures was investigated. The primary goal of this experiment was to determine if the addition of grass to alfalfa could be an effective strategy alone or combined with the use of tolerant cultivars and/or insecticide applications to reduce the effect of potato leafhoppers on alfalfa. The intent is to contribute to the development of an integrated pest management strategy that could reduce the need for insecticides. Section 4.2 (Materials and methods) was initially developed by Philippe Seguin (McGill), Céline Georlette (CDBQ), Huguette Martel (MAPAQ), and Julien Saguez (CEROM), with input from Annie Claessens (AAFC). Fieldwork presented in Section 4.2.2, was done mostly by Xiawei Shi and supervised by P. Seguin at Sainte-Anne-de-Bellevue and by J. Saguez and his team at La Pocatière. Statistical analyses presented in Section 4.2.3, were done by X. Shi and supervised by

P. Seguin. The results, discussions, and conclusions sections were primarily written by X. Shi and revised by P. Seguin.

CHAPTER 4

Potato leafhoppers in alfalfa: Effects of alfalfa-grass proportions, cultivar tolerance level, and insecticides

4.1. INTRODUCTION

The dairy industry, with more than 1.8 million dairy cows, plays a substantial role in Canadian agriculture (Statistics Canada, 2023). Over 70% of dairy cows are in Quebec and Ontario, forming an important forage-based livestock industry (Statistics Canada, 2017). Forages are vital to the dairy industry as they are the primary component of dairy cows diet (Claessens & Biligetu, 2018). Enhancing the nutritive value, yield, and persistence of forage crops is central to ensuring profitability of the dairy industry. Alfalfa (*Medicago sativa* L.), a perennial forage crop, is extensively grown worldwide, primarily as a high yield potential and high-quality forage source for livestock (Seguin et al., 2004). Alfalfa and alfalfa mixtures are grown on over 3 million hectares in Canada, ranking fourth among crops in terms of land area on which it is grown (Statistics Canada, 2021).

There are, however, a variety of abiotic and biotic stresses limiting alfalfa production, including climate change, drought, disease, and insect predation (Singer et al., 2018; Yan et al., 2023), leading producers to spend more money buying hay which may sometimes, consequently, impact farm profitability. The potato leafhopper [*Empoasca fabae* (Harris)), a serious agricultural pest across the eastern US and parts of Canada, adversely affects alfalfa by affecting its yield, nutritive value and winter survival (Sulc et al., 2004). Although historically rare in Quebec, this insect recently becomes a problem, exerting a significant effect on alfalfa production in neighboring regions. There is little information locally on the economic losses associated with the occurrence of this insect in alfalfa, but in Ontario, where it has been observed regularly for nearly 30 years, estimates suggest that it could cause annual losses of up to $250 million (Bullas Appleton et al., 2003). Recently, potato leafhoppers have been calculated to cause alfalfa yield losses, amounting to approximately $500 per hectare every year in some regions of Ontario (Quesnel, 2012).

Each summer, potato leafhoppers migrate northward from the Gulf States to the Midwest and eastern United States, as well as some parts of Canada (Sulc et al., 2004; Chasen et al., 2014). As they do not overwinter in Quebec, infestation levels in one year cannot serve as a reliable predictor of the risk of potato leafhoppers in subsequent years (Chasen et al., 2014). Adults typically make their first appearance in Quebec around the beginning of June (Légaré et al., 2013). Potato leafhoppers can produce several generations per season (i.e., 1 to 3). Both adults and nymphs inflict damage on alfalfa by feeding in the phloem of stems and petioles (Chasen et al., 2014). The damage caused by the introduction of their stylet into the plant tissues and subsequent saliva that elicits a defensive response from the plant, causing a blockage of the vascular tissues which affects the movement of nutrients. The distinctive symptom of a potato leafhopper attack in alfalfa is reduced stem growth and leaf chlorosis, identified as "hopperburn" on leaves, initiating as a yellow "V" shape and spreading outward from the center to the tip (Sulc et al., 2001; Calvin et al., 2013).

Strategies to mitigate the effects of potato leafhoppers include the application of insecticides, the use of tolerant cultivars, and the use of alfalfa-grass mixtures instead of pure alfalfa stands (Chasen et al., 2014). The main strategy currently used to control potato leafhoppers involves applying insecticides combined with scouting, according to application thresholds, which vary by locations. In Quebec, the thresholds are based on those of Ohio and depend on the height of alfalfa (Légaré et al., 2013). Hammond et al. (2014) reported that timely insecticide applications along with regular scouting can significantly reduce potato leafhopper activity, consequently leading to the improved alfalfa yield. Faris et al. (1981) also noted that potato leafhopper feeding resulted in a 17% reduction in average total forage yield during the seeding year and post-seeding year compared to when treated with insecticides.

Cultivars of alfalfa tolerant to potato leafhoppers have been available since 1997 in the United States (Elden and McCaslin, 1997). These cultivars have glandular trichomes on the surface of stems and leaves, limiting the mobility of adults and nymphs of potato leafhoppers and their feeding rate (Sulc et al., 2014; Obermeyer 2020). Many of the cultivars originally developed were more suited to the American Midwest, but cultivars adapted to conditions in the eastern United States and Canada have since become available, including some in Quebec from local seed companies. However, to our knowledge, no study has evaluated these different tolerant cultivars in Quebec in a same comparative trial with traditional susceptible cultivars. In the USA, several studies conducted in the North indicate that the performance of potato leafhopper-tolerant cultivars varies across different regions. Sulc et al. (2001) reported that in the Midwest US, when facing high potato leafhopper populations, potato leafhopper-tolerant cultivars had 50% greater forage yields, compared to susceptible ones. In contrast, out of four years in a trial conducted in New York state, only in one year did potato leafhopper-tolerant alfalfa cultivars yield more than susceptible ones (Hansen et al., 2002). It is noteworthy that in some reports from the USA, when facing low potato leafhopper populations, some tolerant alfalfa cultivars yielded up to 15% less compared to susceptible cultivars (Hansen et al., 2002; Chasen et al., 2013; Wiersma and Thomas, 2016).

Using alfalfa-grass mixtures instead of pure alfalfa may be another method to reduce potato leafhopper populations and the use of insecticides. Introducing grasses into alfalfa can dilute potato leafhopper attacks, because the presence of grasses makes it more challenging for insects to locate alfalfa (Difonzo, 2019). Studies carried out in the USA have shown that adding as little as 9% grasses [smooth bromegrass (*Bromus inermis* Leyss.) or orchardgrass (*Dactylis glomerata* L.)] in alfalfa fields reduced potato leafhopper populations by 4 to 37% compared to pure alfalfa fields (Rode et al., 1997). However, recently, Chasen et al. (2013) reported limited benefit from mixing orchard grass with alfalfa when potato leafhopper populations were low. The impact on potato leafhoppers of growing alfalfa and grasses mixtures seems to be variable and could be due to the proportion of grasses in the mixture, thus, the benefits of adding varying proportions of grass in mixtures with alfalfa remains to be evaluated.

Our objective was to determine whether adding grass to alfalfa could be an effective strategy on its own or in combination with the use of tolerant cultivars and/or insecticide applications, in order to reduce the effect of potato leafhoppers on alfalfa in

Quebec. Our ultimate goal is to minimize the effects of potato leafhoppers on alfalfa, thereby contributing to the development of an integrated pest management strategy that could potentially reduce dependence on insecticides.

4.2. MATERIALS AND METHODS

4.2.1. Sites and treatments description

An experiment was conducted at two contrasted sites in Quebec: Sainte-Anne-de-Bellevue (SAB, 2332 cumulated growing degree days on a 5°C basis [GDD5]), on a Chicot sandy loam (45° 25' 38.0" N lat., 73° 55'45.0" W long.) and La Pocatière (POC, 1846 cumulated GDD5), on a Kamouraska heavy clay soil (47° 21'21.0" N lat., 70° 1' 55.0" W long.). At each of the two sites, treatments evaluated included a combination of two alfalfa cultivars, a potato leafhopper-tolerant cultivar (i.e., WL358LH) or a locally adapted susceptible cultivar (i.e., Dominator), three alfalfa-grass mixture proportions (100:0, 75:25, and 50:50), and two insecticide treatments (i.e., with or without). These combinations resulted in a total of twelve treatments. Two grass species, smooth bromegrass and timothy (*Phleum pretense* L.) were included in mixture treatments. Seeding rates were 14 kg ha^{-1} for alfalfa in pure alfalfa plots (100:0); 10.5 kg ha^{-1} alfalfa, 4.5 kg ha^{-1} smooth bromegrass, and 2.5 kg ha^{-1} timothy in 75:25 plots; 7 kg ha^{-1} alfalfa, 9 kg ha^{-1} smooth bromegrass, and 5 kg ha^{-1} timothy in 50:50 plots. These seeding rates represented percentages of recommended rates on a pure-live seed basis, and not percentages of seed density per surface area (CRAAQ, 2005). Seeding was done at a targeted depth of 10 mm and rows spaced at 18 cm using a Fabro seven-row seeder (Swift Current, SK, Canada) at SAB and a Kincaid seven-row seeder (Haven, KS) at POC. Plots were seeded in the spring of 2021 at SAB (SAB-A) and POC and were harvested in 2021, 2022 and 2023; another set of plots was seeded in 2022 at SAB (SAB-B) and harvested in 2022 and 2023. Depending on the site, plot sizes varied, but were a minimum area of 1.3 × 5 m. Every treatment combination was replicated four times resulting in 48 plots in total at each site (i.e., SAB-A, SAB-B, and POC).

Plots were managed following local recommendations with fertilization being done before seeding based on soil analyses (CRAAQ, 2010). No herbicide treatment was done as most plots included alfalfa-grass mixtures. Plots were treated with insecticides when potato leafhopper populations reached recommended thresholds for treatment (Table 4.1) (RAP, 2020).

The insecticide Matador 120EC [Syngenta Crop Protection Canada, Guelph, ON, Canada; with lambda-cyhalothrin (120 g L^{-1})] was applied at a rate of 83 mL ha each time the potato leafhopper population exceeded the threshold. This resulted in three applications in the seeding year in SAB-A (i.e., 23 June, 23 July, and 11 August 2021), two at SAB-B (i.e., 6 July and 18 July 2022), and one at POC (i.e., 27 July 2021). In post-seeding years, the threshold was reached only once in the first post-seeding year in SAB-A (i.e., 20 July 2022), no applications were thus done at the other sites (i.e., SAB-B or POC). All plots were harvested one or two times in the seeding year and three to four times in post-seeding years, the number of harvests depending on the site and environmental conditions.

The experimental design used was a randomized complete block with split-split-plot restriction and four replicates. Insecticides treatments were assigned to main plots, alfalfa cultivars to subplots, and alfalfa-grass proportions to sub-subplots. Buffer borders 4.5 m wide were seeded with a potato leafhopper-susceptible alfalfa cultivar around each main plot as per Sulc et al. (2014).

4.2.2. Data collection

Plot harvests were done when plants were at the early flowering stage or based on a 450-500 GDD5 interval between harvest. A 0.6 × 5 m strip was harvested using a forage small-plot harvester in each plot to determine forage yields. A 500 g fresh sample was then collected and dried for at least 48 hours at 55 °C in a forced-air oven to determine forage yields on a dry matter basis. At each harvest, alfalfa height was also measured using ten randomly selected plants in each plot to assess the effect of potato leafhopper on alfalfa growth and development. The vegetation in a permanent quadrat of 0.5 × 0.5 m^2 located in each plot was also harvested using scissors and collected samples were separated by hand into alfalfa, grasses, and weeds components, each being dried for at least 48 hours at 55 °C to determine yield contributions of each component on a dry matter basis.

Potato leafhopper populations (i.e., sum of adults and nymphs) were monitored weekly at each site in each of the main plots from June to August, using an established protocol (RAP, 2020). Briefly, 10 sweeps were performed per main plot in each replicate using a net with a 30-40 cm diameter avoiding the first meter on each side of the main plots.

4.2.3. Statistical analyses

Data were analyzed using a three-way analysis of variance (ANOVA) with PROC GLM of the SAS software (SAS Institute, 2014). Replicates for each site were considered a random effect while insecticide, cultivar and alfalfa-grass proportion effects were considered fixed. When interactions were significant, data were further analyzed using one-way ANOVA. Differences between treatments means were determined using the Least Significant Difference (LSD) test at the significance level of $\alpha = 0.05$. Only significant effects ($P < 0.05$) are discussed herein.

4.3. RESULTS

4.3.1. Potato leafhopper populations and insecticide applications

In the seeding year, potato leafhopper populations reached the threshold for insecticide treatments three times at SAB-A, twice at SAB-B, and once at POC. Populations observed reached peaks of 22, 41, and 50 insects per ten sweeps at SAB-A, SAB-B and POC, respectively (Figure 4.1). Based on populations observed, using local recommendations (Table 4.1), one insecticide application was applied (23 June 2021) before the first harvest and two more (23 July and 11 August 2021) before the second harvest at SAB-A, while at SAB-B, two applications were made (6 July and 18 July 2022) before the first harvest. Finally, at POC, only one application was done (27 July 2021) before the only harvest made (Figure 4.1). Differences in potato leafhopper populations between insecticide treatments were observed at three dates at SAB-A (29 June, 2 August, and 10 August 2021), four at SAB-A (28 June, 7 July, 14 July, and 21

July 2022), and three at POC (2 August, 10 August and 17 August 2021) (Figure 4.1).

In the first post-seeding year, only once at SAB-A did potato leafhopper populations exceeded the threshold, reaching a peak of 13 insects per ten sweeps. Thus one insecticide application was applied (20 July 2022) before the third harvest. At both other sites (i.e., POC and SAB-B), potato leafhopper populations remained low, ranging from 0 to 2 and 0 to 3 insects per ten sweeps, respectively, thus never reaching the threshold level required for insecticide applications (Figure 4.1).

In the second post-seeding year, at SAB-A and POC, potato leafhopper populations always remained below the application threshold for insecticide treatments, ranging from 0 to 4 and 0 to 0.3 insects per ten sweeps, respectively, thus no applications were made (Figure 4.1).

4.3.2. Plant height and forage yield in the seeding year

In the seeding year, there were two forage harvests in SAB-A and SAB-B, and one in POC. In SAB-A, an insecticide × cultivar interaction was observed for alfalfa yield at the first harvest (Data not shown, $P < 0.05$; see Table A1), while an insecticide × cultivar × alfalfa:grass mixture proportion interaction was significant at the second harvest for alfalfa yield as well ($P < 0.05$). An insecticide main effect for alfalfa height at the second harvest was also observed ($P < 0.01$). In SAB-B, an insecticide × mixture proportion interaction for weeds mass at the second harvest was observed (Data not shown, $P < 0.05$; see Table A4). Finally, at POC, differences in yields between insecticide treatments, cultivars, or mixture proportions were minimal in the seeding year, only a cultivar main effect being observed for alfalfa height at the first harvest (Data not shown,

$P < 0.05$; see Table A6).

In SAB-A, with high potato leafhopper populations, the insecticide × cultivar interaction for alfalfa yield at the first harvest illustrated that for Dominator (a potato leafhopper-susceptible cultivar), one insecticide application increased alfalfa yield by 120% (Figure 4.2). In contrast, WL358LH (a potato leafhopper-tolerant cultivar) did not respond to the insecticide application with alfalfa yield being comparable if treated or not. The insecticide × cultivar × mixture proportion interaction for alfalfa yield at the second harvest, revealed that when Dominator was seeded without grasses, three insecticide applications increased yield by 41% compared to the untreated 100% alfalfa plots (Figure 4.3). In contrast, the application of insecticides had no effect on alfalfa yields of Dominator when mixed with grasses in either proportion. In the case of WL358LH, the applications of insecticide did not impact alfalfa yield of any of the mixture treatments. It is important to note when looking at responses to mixture treatments that proportions of alfalfa and grasses in mixtures did not yield the targeted proportions of 75:25 and 50:50. For Dominator, alfalfa and grass yields at the second harvest in 50:50 plots were 1298 and 70 kg ha^{-1}, respectively, and thus alfalfa accounted for 95% of the total forage yield (Data not shown). Alfalfa and grass yields in the 75:25 plots were 1079 and 48 kg ha^{-1}, alfalfa representing 96% of the total forage yield (Data not shown). The very small differences in alfalfa:grass proportions between the 50:50 and 75:25 treatments mostly explain why there is no difference between these treatments. The only other treatment response observed in the seeding year was, at the second harvest, three insecticide applications resulted in a 37% increase in the average alfalfa plant height compared to when no insecticide was applied (Data not shown, $P < 0.01$; see Figure A27).

In SAB-B, the insecticide × mixture proportion interaction for weeds mass at the second harvest reflected that the response to insecticide applications with high potato leafhopper populations varied depending on the alfalfa:grass proportion ($P < 0.05$). In the 75:25 and pure alfalfa plots, insecticide applications were associated with 38% and 52% lower weeds mass, respectively, compared to when no insecticide was applied (Figure 4.4). In contrast, plots of the 50:50 treatment did not respond to insecticide applications. When comparing mixture treatments not treated with insecticides and thus subjected to potato leafhopper predation, mixing alfalfa with grasses (i.e., 75:25 and 50:50 treatments) reduced weeds mass by 40% compared to pure alfalfa plots, while there was no difference between 50:50 and 75:25 treatments. As mentioned earlier for SAB-A, our achieved alfalfa:grass proportions differed from our targeted ones. The proportions observed in the 50:50 treatment averaged 70% alfalfa and 30% grasses, while it was 74% alfalfa and 26% grasses in the 75:25 treatment. As we can see, the achieved alfalfa;grass proportions in both treatments were comparable, thus maybe explaining the lack of difference between these two treatments.

Finally, at POC, differences between cultivars were observed for plant height at the only harvest made with Dominator being 7% taller than WL358LH, demonstrating a lack of advantage for the tolerant cultivar (Data not shown, $P < 0.05$; see Figure A28).

4.3.3. Plant height and forage yield in the post-seeding years

In the first post-seeding year, there were four forage harvests in SAB-A, three in SAB-

B and three in POC. In SAB-A, numerous treatment interactions were observed (Data not shown, $P < 0.05$; see Table A2). Insecticide × cultivar interactions for total forage yield at the first harvest, alfalfa yield at the first and second harvests, annual total forage yield and annual alfalfa yield were observed. An insecticide × cultivar interaction × mixture proportion was observed for alfalfa height at the third harvest. Main effects were also observed, including cultivar main effects for total forage yield and alfalfa yields at the third and fourth harvests and for alfalfa height at the second and fourth harvests. Finally, an insecticide main treatment effect for alfalfa height at the first harvest was observed. At POC, cultivar main effects were observed for annual total forage yield, annual alfalfa yield, and total forage and alfalfa yields at the first and third harvests (Data not shown, $P < 0.05$; see Table A7). Insecticide × cultivar interactions for total forage and alfalfa yields at the third harvest were also observed. Finally, in SAB-B, differences between treatments were minimal, no treatment main effects or interactions being observed (Data not shown, $P > 0.05$; see Table A5).

It is important to note that at SAB-A, insecticide applications made in the seeding year had residual effects at the first two harvests, while in contrast, the insecticide application done before the third harvest in the first post-seeding year had minimal effects. The residual effect of the insecticide was although only observed for Dominator, the potato leafhopper-susceptible cultivar, not for WL358LH, the tolerant cultivar. The differential residual response is illustrated by the insecticide × cultivar interactions observed for total forage and alfalfa yields (Figure 4.5; $P < 0.05$). At the first harvest, total forage yield of Dominator was increased by 17% by insecticides applied in the previous year, while no differences between insecticide treatments were observed for WL358LH. When untreated with insecticides, WL358LH plots yielded 14% more than Dominator, suggesting that the potato leafhopper tolerance trait conferred an advantage even in the post-seeding year before being challenged again by insects. The insecticide × cultivar interaction for alfalfa yield at the first and second harvest illustrated the same differential response of cultivars to insecticide applications done in the previous year (Data not shown, $P < 0.05$; see Figures A29-30). Indeed for Dominator, insecticides increased alfalfa yield at the first and second harvests by 19 and 20%, respectively, while no response to insecticide applications was observed for WL358LH. A small positive effect of insecticide (i.e., 3%) was also observed for alfalfa height at the first harvest across cultivars (Data not shown, $P < 0.05$; see Figure A31). Insecticide × cultivar interactions observed for yields at the first and second harvests were reflected in the annual total forage and annual alfalfa yields ($P < 0.05$). Again for Dominator, insecticide applications increased annual total forage and alfalfa yields by 20 and 17%, respectively, while WL358LH did not respond to insecticide applications made (Data not shown, $P < 0.05$; see Figures A32-33). In contrast, no response to the insecticide application made before the third harvest was observed for either total forage or alfalfa yield at the third and fourth harvests (Data not shown). A response was observed but only for plant height at the third harvest as demonstrated by an insecticide × cultivar × mixture proportion interaction (Data not shown, $P < 0.01$; see Figure A34). For Dominator, insecticide use increased alfalfa plant height in pure alfalfa plots by 31%, while alfalfa in 50:50 and 75:25 plots did not respond to insecticide treatments. In contrast, WL358LH did not respond to insecticide treatments for any of the mixture treatments. This three-way interaction suggested that addition of grasses to alfalfa or use of tolerant cultivars can reduce stress exerted by potato leafhopper on alfalfa in the post-seeding years, but stress was insufficient to translate into a yield response. Overall differences between cultivars were, however, observed for yield and height, regardless of the insecticide or mixture treatments, Dominator performing better than WL358LH. Total forage yield of Dominator plots was 10 and 25% higher than those of WL358LH, at the third and fourth harvests, respectively (Data not shown, $P < 0.01$; see Figures A35-A36). Similarly, alfalfa yield of Dominator was 10 and 26% higher than that of WL358LH, at the third and fourth harvest, respectively (Data not shown, $P < 0.01$; see Figures A37-A38). Finally, Dominator was 5% and 15% taller than WL358LH, at the second and fourth harvest, respectively (Data not shown, $P < 0.05$; see Figures A39-40).

At POC, differences in forage yield between insecticide treatments were limited in the first post-seeding year, only being reflected in insecticide × cultivar interactions for total forage and alfalfa yields at the third harvest (Data not shown, $P < 0.05$; see Figures A41-A42), which illustrate a residual effect of an insecticide application done in the seeding year, the response differing between cultivars. In contrast to the residual response observed in SAB-A, the response observed at POC was for WL358LH, not Dominator, for WL358LH, the insecticide application resulted in increases of 5 and 7% in total forage and alfalfa yields, respectively, while Dominator did not respond to the insecticide application made.

Otherwise, similar to the results in SAB-A, under a lower insect pressure, Dominator performed better at POC than WL358LH in terms of total forage and alfalfa yields at the first and third harvests. Annual total forage and annual alfalfa yields of Dominator were 7 and 9% higher, respectively, than those of WL358LH (Data not shown, $P < 0.01$; see Figures A43-A44). In addition, total forage yield of Dominator was 12 and 5% higher than that of WL358LH at the first and third harvest, respectively (Data not shown, $P < 0.05$; see Figures A45-A46). Finally, alfalfa yield of Dominator was 16 and 5% higher than that of WL358LH at the first and third harvests (Data not shown; $P < 0.05$; see Figures A47-A48).

In the second post-seeding year, there were three forage harvests in SAB-A and two at POC under low potato leafhopper pressure (no insecticide application at either site). In SAB-A, a residual insecticide main treatment effect for alfalfa height was observed at the second harvest (Data not shown, $P < 0.05$; see Table A3), while a cultivar main treatment effect for alfalfa height was observed at the first and third harvests. At POC, cultivar main treatment effects for annual total forage yield, annual alfalfa yield, total forage and alfalfa yields at the first and second harvests, and alfalfa height at the second harvest were observed (Data not shown, $P < 0.05$; see Table A8).

In SAB-A, under low insect pressure, insecticides applied in the previous years had a small apparent effect that increased alfalfa plant height at the second harvest by 4% (Data not shown; $P < 0.05$; see Figure A49). Also, Dominator was 9 and 8% taller than WL358LH at the first and third harvests, respectively (Data not shown; $P < 0.05$; see Figures A50-A51). Thus overall, treatment effects in the second post-seeding year were minimal.

At POC, all significant effects observed reflected that, under low insect pressure, Dominator had an overall better performance compared to WL358LH, no difference between insecticide treatments applied in previous year being observed, except for an insecticide × cultivar interaction for alfalfa height at the first harvest. Annual total forage and annual alfalfa yields of Dominator were 18 and 17%, respectively, higher than those of WL358LH (Data not shown, $P < 0.01$; see Figures A52-A53). Similarly, total forage yield of Dominator at the first and second harvests was 22 and 11% higher than that of WL358LH, respectively (Data not shown, $P < 0.01$; see Figures A54-A55). The same trend was also observed for alfalfa yield at the first and second harvest, the yield of Dominator being 21 and 11% higher than that of WL358LH (Data not shown, $P < 0.01$; see Figures A56-A57). In terms of alfalfa height, in untreated plots Dominator was 9% taller than WL358LH at the first harvest, while the average alfalfa plant height of Dominator was comparable to that of WL358LH when treated with insecticides in the seeding year (Data not shown, $P < 0.05$; see Figure A58). Finally, Dominator was 15% taller than WL358LH at the second harvest (Data not shown, $P < 0.01$; see Figure A59).

4.4. Discussion

High populations of potato leafhoppers that required insecticide applications were observed in the seeding year at the three sites. However, in post-seeding years, the populations were generally low and did not reach the application threshold, except for once in SAB-A in the first post-seeding year. Potato leafhopper populations observed in our experiment were not consistent over these three growing seasons, which is in accordance to our observations for another experiment conducted concurrently and described in Chapter 3. When potato leafhopper populations were high, insecticide applications and timely harvests were effective strategies to reduce populations, as reported in Chapter 3.

In the seeding year, in SAB-A, insecticide applications increased alfalfa yield of the potato leafhopper-susceptible cultivar (i.e., Dominator) at the first harvest, while the alfalfa yield of the potato leafhopper-tolerant cultivar (i.e., WL358LH) was constant whether plots were treated with the insecticide application or not (Figure 4.2). Such response to insecticides in high potato leafhopper conditions of a susceptible cultivar is in agreement with Sulc et al. (2015), whom reported that for the total alfalfa yield of all harvests over three growing seasons, insecticide applications increased the total alfalfa yield of potato leafhopper-susceptible cultivars by 37% compared to that when not treated. In contrast, they observed a smaller response to insecticide applications of tolerant cultivars, with 6% in yield compared to that when not treated. These results also agree with Sulc et al. (2001), whom reported that the advantage of insecticides on alfalfa yield was greater for potato leafhopper-susceptible cultivars than tolerant ones. The differential response of the susceptible and tolerant cultivars to insecticides demonstrates the advantage provided by tolerant cultivars; their use may reduce the need for insecticide applications.

It is also important to note that in the seeding year at the second harvest, again in SAB-A, insecticide applications increased alfalfa yield of Dominator, as observed at the first harvest, but only in pure alfalfa plots (Figure 4.3). In contrast, alfalfa yield in 50:50 and 75:25 plots of Dominator and all the plots of WL358LH, did not respond to insecticide applications. These results indicate that both the addition of grasses to alfalfa field even in very small proportions (i.e., 5 and 4% grass in 50:50 and 75:25 alfalfa plots, respectively) and the use of a potato leafhopper-tolerant cultivar can protect alfalfa from damage by insects. The advantage provided by the tolerant cultivars was also illustrated by the above reported differential response of cultivars to insecticides in the seeding year. Similarly, McCaslin (1998) reported that when facing high potato leafhopper pressure, potato leafhopper-tolerant cultivars yielded more than susceptible ones. Sulc et al. (2001) reported that in the presence of large potato leafhopper populations, in plots not treated with insecticide, the annual alfalfa yield of potato leafhopper-tolerant cultivars was 1.0 to 1.2 Mg ha^{-1} higher than that of susceptible cultivars. In terms of alfalfa:grass mixture, Hansen et al. (2006) reported that for potato leafhopper susceptible cultivars, alfalfa-grass mixture plots had lower insect populations, compared to pure alfalfa plots, which mostly indicated that fewer insect feedings in alfalfa can help increase the alfalfa yield. Furthermore, in the experiment in Michigan by Roda et al. (1997), alfalfa plots containing 9% grasses reduced potato leafhopper populations by 4 to 37% compared to pure alfalfa plots.

In addition to their effect on alfalfa yield, insecticide applications indirectly contributed to reducing weeds mass in 75:25 and pure alfalfa plots compared to when not treated, while weeds mass in 50:50 alfalfa plots did not respond to insecticide applications (Figure 4.4). Also in plots not treated with insecticide, there were fewer weeds in 50:50 and 75:25 alfalfa plots compared to pure alfalfa plots. Such response most likely reflects that insecticide-untreated alfalfa were stressed by potato leafhopper feeding and thus were less competitive with weeds. The response of grasses most likely reduced the effect of potato leafhoppers by reducing potato leafhopper populations in plots, although this could not be confirmed as insect populations were determined per main plots and not in each individual plots. Difonzo (2019) reported that adding grasses into alfalfa can mitigate potato leafhopper attacks on alfalfa, as the grass presence creates a more challenging environment for insects to locate alfalfa in the field, reduced potato leafhopper damage most likely contributes to the stronger establishment of alfalfa, enhancing its resilience against weeds.

In SAB-A, insecticide applications made in the previous year and once in the first post-seeding year increased annual total forage and annual alfalfa yields of Dominator compared to when not treated, while there was no response of WL358LH to insecticide applications (Data not shown; see Figures A32-A33). It is, however, important to note that these responses of annual yields to insecticides mostly reflected a residual response to insecticide applications done in the seeding year rather than to the one application done in the post-seeding year before the third harvest, as no yield response to the insecticide was observed at either the third or fourth harvest, but only at the first two harvests. These results are in agreement with Hansen et al. (2002), who reported a carryover effect of severe potato leafhopper feeding in the seeding year on alfalfa yields in the post-seeding year in two of three trials. With no potato leafhopper observed prior the first post-seeding year harvest, alfalfa yields were significantly greater for potato leafhopper-tolerant alfalfa cultivars than susceptible ones. Large populations of potato leafhoppers and the ensuing damage from their feeding tends to cause decreased alfalfa yields in subsequent harvests or years, disrupting the transfer of photosynthetic products to the root and crown tissues of alfalfa. (Lamp et al., 2001; Chasen et al., 2014).

In post-seeding years in SAB-A and POC, in the absence of potato leafhopper populations, Dominator performed better than WL358LH, in terms of alfalfa plant height, total forage yield and alfalfa yield. Our results were similar to the experiment of McCaslin (1998), under the low insect pressure or when treated with insecticides, the forage yield of potato leafhopper-tolerant cultivars was lower than that of potato leafhopper-susceptible cultivars. Thus, if a tolerant cultivar could perform better than a susceptible cultivar in high potato leafhopper situations and even reduce the need for insecticide applications, in low potato leafhopper situations its productivity was lower. Such results suggest that the use of tolerant cultivars should be restricted to areas where populations of potato leafhoppers are consistently high.

4.5. Conclusions

Populations of potato leafhopper exhibit varied from year to year and across sites. In the current study, low populations were overall observed in two out of three years, necessitating multiple insecticide applications in the seeding year at all three sites, but only once in the first post-seeding year at one site. The combination of insecticide applications based on weekly scouting and thresholds, as well as timely harvests were both equally effective to reduce potato leafhopper populations. At one out of three sites, insecticides directly affected yields positively in the seeding year of only the potato leafhopper-susceptible cultivar, on which it also had residual effects in the first post-seeding year. The advantages of using a potato leafhopper-tolerant cultivar were overall limited across years and sites, even having lower yields in post-seeding years compared to a susceptible cultivar in some low potato leafhopper environments. The effect of growing alfalfa in mixtures with grasses was minimal, possibly because the contribution of grasses we achieved were small. The addition of small percentages of grasses into alfalfa fields although contributed to reducing weeds at one out of three sites in the seeding year, this effect was comparable to that achieved with the use of insecticides. As potato leafhopper populations varied considerably across environments, and as they were low in most post-seeding year, the use of potato leafhopper-tolerant cultivars, insecticides, and alfalfa-grass mixtures, need to be validated across a greater range of environments in Quebec before recommendations can be made.

Table 4.1. Insecticide intervention threshold according to the average number of potato leafhoppers and alfalfa plant height.

Plant height (cm)	Average number of potato leafhoppers per 10 sweeps
Under 15	4
15-19	6
20-24	8
25-29	10
30-34	12
35-39	14
40-44	16
45-49	18
50 and above	20

Adapted from RAP (2020).

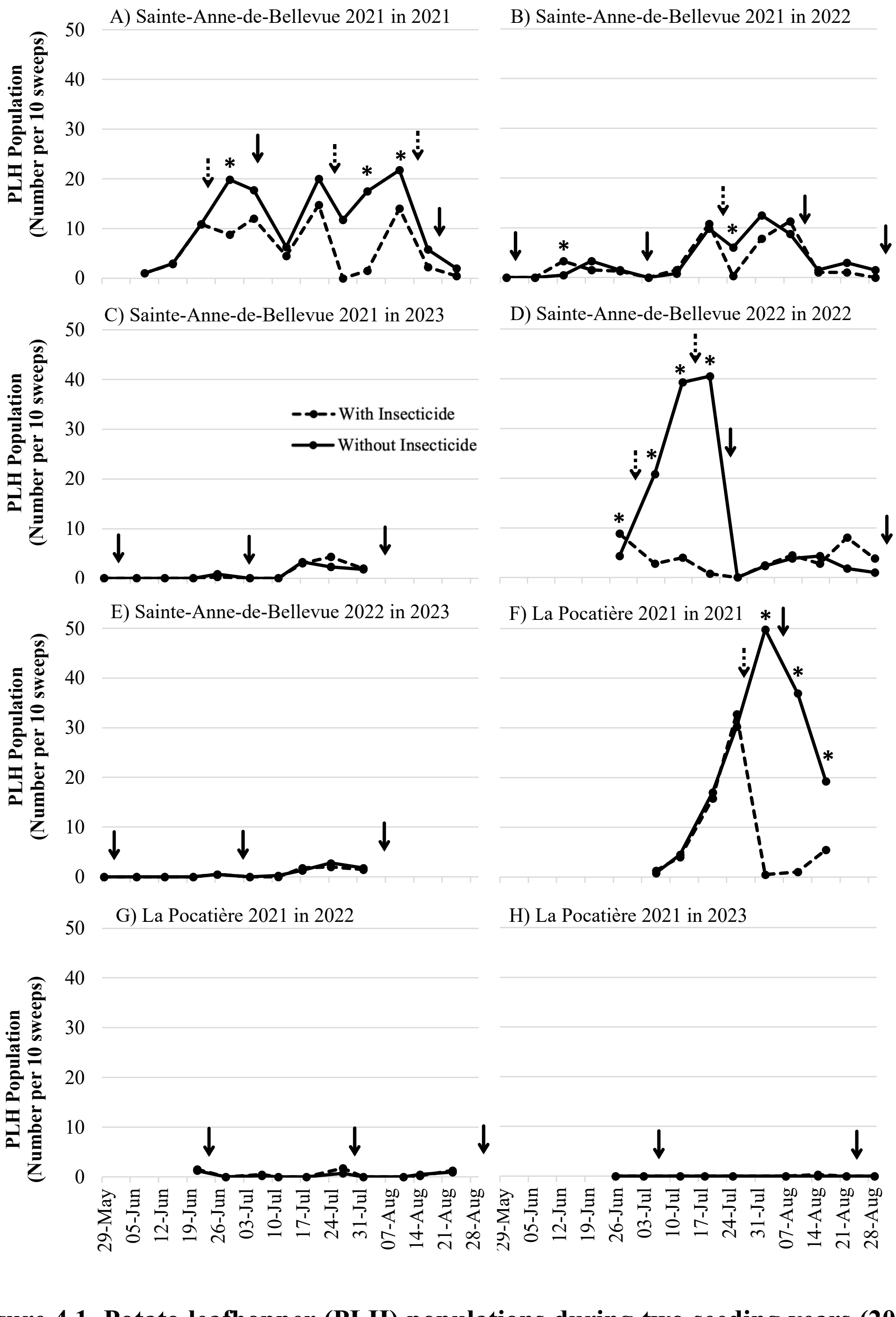

Figure 4.1. Potato leafhopper (PLH) populations during two seeding years (2021 and 2022), as well as first two post-seeding years (2022 and 2023) at two contrasted sites in Quebec, Canada and submitted to two insecticide treatments (with and without)

*** = Differences between insecticide treatments (P < 0.05) at specific dates; ↓ = Forage harvests; ⇣ = Insecticide applications.**

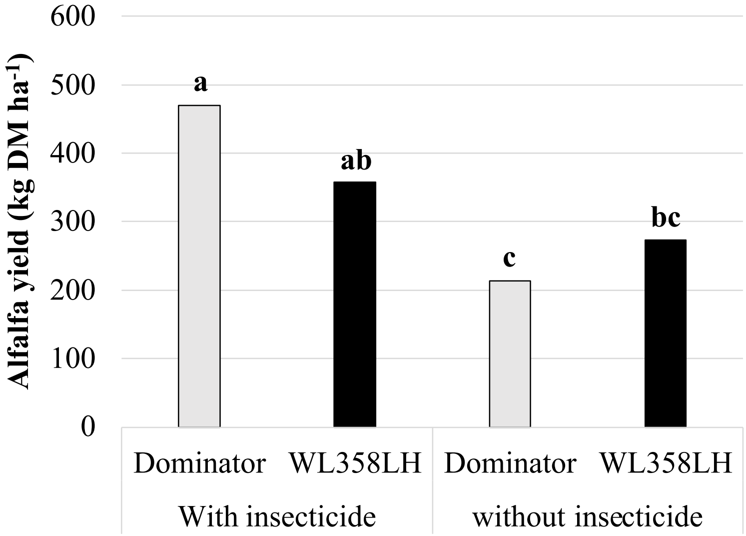

Figure 4.2. Alfalfa yield at the first harvest in the seeding year (2021) of two alfalfa cultivars (Dominator, potato leafhopper susceptible; WL358LH, potato leafhopper-tolerant) submitted to two insecticide treatments (with and without) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Two insecticide applications were done before the harvest. Values are the average of three alfalfa-grass proportions (50:50, 75:25 and 100:0).

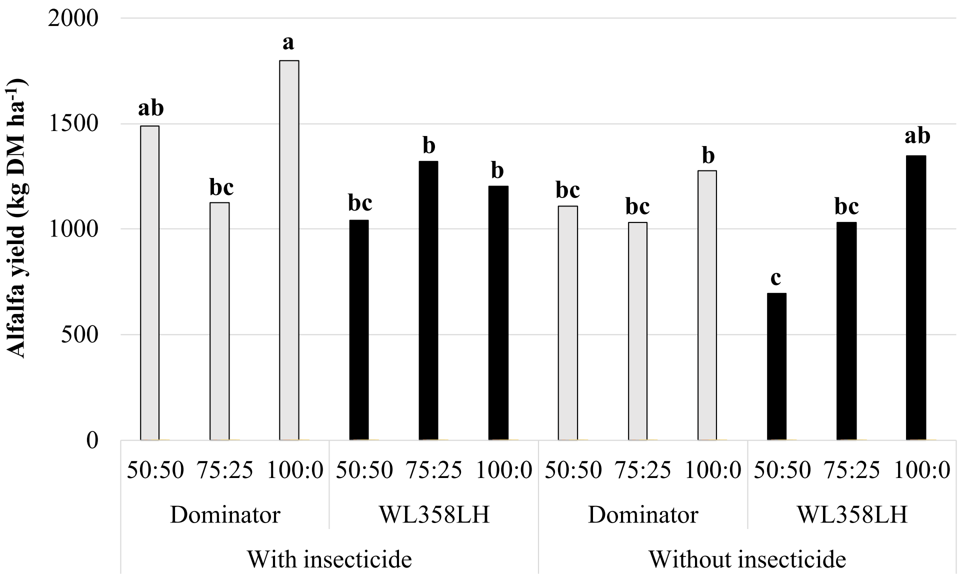

Figure 4.3. Alfalfa yield at the second harvest in the seeding year (2021) of the combination of two alfalfa cultivars (Dominator, potato leafhopper-susceptible; WL358LH, potato leafhopper-tolerant), two insecticide treatments (with and without), and three alfalfa-grass proportions (50:50, 75:25 and 100:0) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Three insecticide applications were applied before the second harvest.

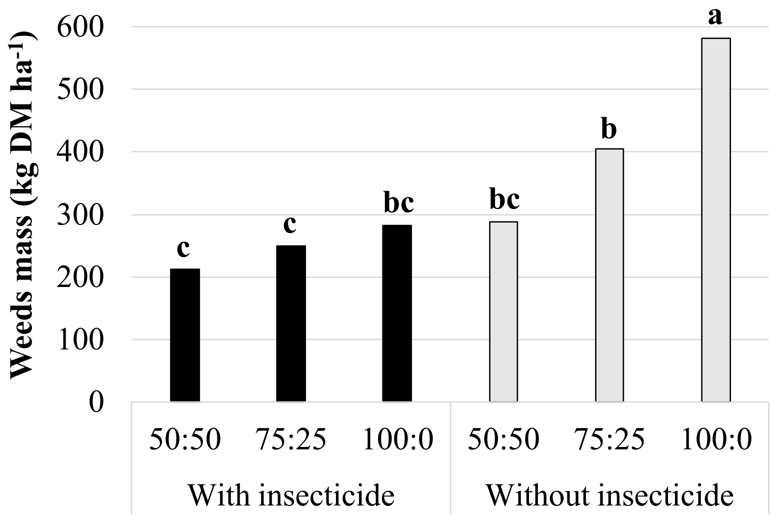

Figure 4.4. Weeds mass at the second harvest in the seeding year (2022) of three alfalfa-grass mixtures (50:50, 75:25 and 100:0) and two insecticide treatments (with and without) at Sainte-Anne-de-Bellevue (SAB-B), QC, Canada. Means followed by different letters are significantly different (P < 0.05). Two insecticide applications were applied before the second harvest. Values are the average of two alfalfa cultivars (Dominator, potato leafhopper-susceptible; WL358LH, potato leafhopper-tolerant).

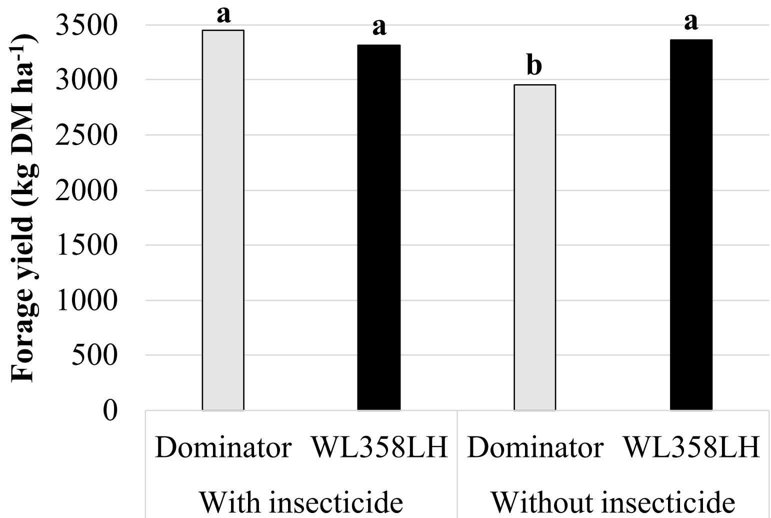

Figure 4.5. Total forage yield at the first harvest in the first post-seeding year (2022) of two alfalfa cultivars (Dominator, potato leafhopper-susceptible; WL358LH, potato leafhopper-tolerant), submitted to two insecticide treatments (with and without) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada. Means followed by different letters are significantly different (P < 0.05). No insecticide application was applied before the first harvest. Values are the average of three alfalfa-grass proportions (50:50, 75:25 and 100:0).

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Alfalfa (*Medicago sativa* L.) has been regarded as the "Queen of forages", due to its high yield potential and nutritive value (Barnes et al., 1988; Seguin et al., 2004). However, the potato leafhopper [PLH, *Empoasca fabae* (Harris)], a serious agricultural pest in the USA (Nielsen et al., 1990), has become an emerging problem affecting alfalfa production in Quebec. Our objective was to assess different management strategies with the goal of mitigating yield losses attributed to this pest.

Potato leafhopper populations fluctuated over the three years of experimentation. Populations that exceeded the locally recommendation threshold for insecticide applications were observed in all five sites-years in the seeding year, but only in one site-year in post-seeding years across both experiments and all sites. Under these conditions, insecticide applications based on weekly scouting and recommended thresholds, and timely harvests were both considered effective strategies to lower potato leafhopper populations.

Considering results from our two experiments, in the seeding year with high potato leafhopper populations in five of the five sites-years, the use of insecticides and the use of tolerant cultivars only positively affected alfalfa yield at both of two harvests at one site (i.e., SAB-A in experiment 2). The use of insecticides also positively affected CP concentrations at two sites (SAB and POC in experiment 1), although the response was greater for susceptible cultivars at one of the two sites. Only at one site (SAB in experiment 1) did some tolerant cultivars reduced the negative effect of potato leafhoppers on CP concentration. In the case of the strategy of adding grasses to alfalfa in mixtures, it positively affected alfalfa yield at one site (i.e., SAB-A in experiment 2) at the second harvest when used with a susceptible cultivar. A lack of consistent positive response to either insecticides or the use of potato leafhopper-tolerant cultivars have previously been reported by others, for example, in New York State (Hansen et al., 2002). This limited response in particular to insecticides suggest that maybe the treatment threshold levels for the seeding year might have to be re-examined for Quebec.

In the first post-seeding year, a positively residual response to insecticides applications made in the previous year was observed in two of five sites-years (i.e., SAB in experiment 1 and SAB-A in experiment 2), alfalfa and in some cases total forage yields being greater when insecticides were applied compared to when they were not applied. In one site-year (SAB-A in experiment 2), a positive response to the use of a tolerant cultivar was also observed. We observed no response to the use of insecticide, of a tolerant cultivar, or of adding grasses in the only situation when high potato leafhopper populations were observed in the post-seeding year. In the second post-seeding year, no residual responses were observed when potato leafhopper populations were low. It is important to note that in low potato leafhopper conditions, the use of tolerant cultivar was associated with lower alfalfa yields in some environments. Lower performances of some potato leafhopper resistant cultivars compared to susceptible ones were previously reported in other regions as well (McCaslin, 1998). It is possible that the overall limited response we observed to the addition of grasses to alfalfa was attributed to the small contribution of grasses to total yield we observed and which varied between 4 and 25%.

In conclusion, before recommendations can be made regarding which management strategy to favor among the three we evaluated herein, it is necessary to conduct more experiments in a wider range of environments. The effectiveness of the use of potato leafhopper-tolerant alfalfa cultivars, insecticide applications, and the potential mixture proportion of alfalfa and grass should continue to be evaluated across a broader range of environments in Quebec, especially given that we had few occurrences of high potato leafhopper populations in post-seeding years.

The hypotheses we established at the onset of experiment were only partially confirmed.

(1) Potato leafhoppers negatively impact forage yield and nutritive value of alfalfa.

Partially confirmed. For forage yields, in the seeding year with high potato. leafhopper conditions, forage yield was reduced in one site-year. In the first post-seeding year with the absence of potato leafhoppers, the small carryover effects reduced forage yield in two of five sites-years. In terms of alfalfa nutritive value, overall, only in the seeding year reductions were observed, CP concentration being reduced in one-site-year.

(2) Potato leafhopper tolerant alfalfa cultivars are less impacted by potato leafhopper and their use can reduce the need for insecticides.

Partially confirmed. The performance of potato leafhopper-tolerant alfalfa cultivars evaluated in our experiments varied depending on insect populations and environmental conditions. In only few limited environments they did reduce the negative effects of potato leafhoppers.

(3) Mixing grasses with alfalfa reduces potato leafhopper injury in alfalfa, which in turn will increase total forage yields compared to pure alfalfa stands.

Not confirmed. In the seeding year, the addition of small proportions of grass to alfalfa increased alfalfa yield in one of three years-sites by reducing potato leafhopper damage, but this was not reflected in total forage yields.

CHAPTER 6

RECOMMENDATIONS FOR FUTURE RESEARCH

The results of our project demonstrated that the use of insecticides based on weekly scouting and thresholds, as well as timely harvests during growing seasons both can effectively reduce potato leafhopper populations. Alternatively, the use of potato leafhopper-tolerant alfalfa cultivars and the addition of grass to alfalfa fields can reduce the negative effects of potato leafhoppers have on alfalfa, however, the effectiveness of these strategies were overall minimal and varied from year to year and across sites. Therefore, further research should continue to evaluate the use of different potato leafhopper-tolerant cultivars and alfalfa-grass mixtures in a wider range of environments in Quebec to identify the best management strategies to control this insect and develop a sustainable integrated pest-management strategy.

In addition, since low potato leafhopper populations were observed in a majority of sites-years of our project and as we observed limited response to treatments in the seeding year with high populations, the insect threshold defined for insecticide treatments could be reassessed in order to possibly be adapted to the environmental condition of Quebec.

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APPENDICES

Table A1. P values of three treatments main effects, [i.e., two insecticide treatments (with and without), two alfalfa cultivars (Dominator, potato leafhopper-susceptible; WL358LH, potato leafhopper-tolerant), and three alfalfa-grass proportions (50:50, 75:25 and 100:0)] and their interactions for annual total forage and alfalfa yields and annual weeds mass, as well as total forage and alfalfa yields and weeds mass at the first and second harvests in the seeding year (2021) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada.

Sainte-Anne-de-Bellevue 2021 in 2021												
	Annual				First harvest				Second harvest			
Factors	Total forage yield	Alfalfa yield	Grass yield	Weeds mass	Total forage yield	Alfalfa yield	Grass yield	Weeds mass	Total forage yield	Alfalfa yield	Grass yield	Weeds mass
I†	0.1666	0.2407	0.6766	0.4206	0.2261	0.1943	0.6745	0.5370	0.1541	0.2984	0.9311	0.3514
C‡	0.0758	0.1074	0.4767	0.5334	0.9918	0.3259	0.4730	0.3732	0.1120	0.1282	0.1187	0.7275
P§	0.2229	0.0081	<0.0001	0.8649	0.4343	0.6981	0.0001	0.7014	0.0220	0.0006	<0.0001	0.8796
I × C	0.2754	0.2016	0.7150	0.3597	0.0903	0.0130	0.2613	0.3196	0.7034	0.4819	0.1664	0.4247
I × P	0.8267	0.6583	0.3038	0.2344	0.7206	0.9988	0.6900	0.3363	0.7168	0.4461	0.3207	0.1386
C × P	0.3445	0.0404	0.8493	0.2463	0.0942	0.4744	0.8850	0.0446	0.1956	0.0077	0.6176	0.3851
I × C × P	0.6015	0.1327	0.7988	0.2713	0.5269	0.8345	0.7080	0.4427	0.3735	0.0286	0.5980	0.2322

† I, Insecticide treatments;
‡ C, Cultivars;
§ P, Alfalfa:grass proportions.

Table A2. P values of three treatments main effects [i.e., two insecticide treatments (with and without), two alfalfa cultivars (Dominator, potato leafhopper-susceptible; WL358LH, potato leafhopper-tolerant), and three alfalfa-grass proportions (50:50, 75:25 and 100:0)] and their interactions for annual total forage and alfalfa yields and annual weeds mass, as well as total forage and alfalfa yields and weeds mass at the first, second, third and fourth harvests in the first post-seeding year (2022) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada.

Sainte-Anne-de-Bellevue 2021 in 2022												
	Annual				First harvest				Second harvest			
Factors	Total forage yield	Alfalfa yield	Grass yield	Weeds mass	Total forage yield	Alfalfa yield	Grass yield	Weeds mass	Total forage yield	Alfalfa yield	Grass yield	Weeds mass
I[†]	0.3382	0.3132	0.8408	0.2260	0.2135	0.1782	0.7483	0.3161	0.4893	0.4239	0.9026	0.2700
C[‡]	0.2035	0.1856	0.8563	0.2171	0.1092	0.4561	0.0360	0.2650	0.8726	0.5442	0.1476	0.2988
P[§]	0.0832	0.0002	<0.0001	0.7134	0.2034	0.0027	<0.0001	0.7100	0.0618	0.0004	<0.0001	0.8880
I × C	0.0152	0.0203	0.6053	0.3844	0.0093	0.0146	0.2420	0.3159	0.0526	0.0290	0.2534	0.5629
I × P	0.3516	0.5732	0.0646	0.9115	0.6850	0.8376	0.1591	0.6562	0.5303	0.7725	0.0878	0.8050
C × P	0.0991	0.0282	0.0319	0.3580	0.0966	0.0114	0.0027	0.4435	0.1521	0.1738	0.5592	0.3353
I × C × P	0.3259	0.2998	0.8237	0.5669	0.9592	0.8241	0.5444	0.4508	0.3618	0.4480	0.8098	0.3765

	Third harvest				Fourth harvest			
I	0.3742	0.3936	0.6222	0.4546	0.2656	0.2693	0.4016	0.0821
C	0.0089	0.0070	0.6412	0.5972	0.0074	0.0051	0.8174	0.9362
P	0.2558	0.0006	<0.0001	0.0122	0.4030	0.0542	<0.0001	0.5955
I × C	0.0970	0.1410	0.4911	0.9674	0.3022	0.2472	0.8763	0.8398
I × P	0.3385	0.3848	0.4599	0.6835	0.1314	0.2429	0.5636	0.9047
C × P	0.2347	0.1507	0.5580	0.7382	0.0246	0.0340	0.4547	0.3092
I × C × P	0.0610	0.0188	0.7473	0.9067	0.0597	0.0774	0.7436	0.3691

[†] I, Insecticide treatments;
[‡] C, Cultivars;
[§] P, Alfalfa:grass proportions.

Table A3. P values of three treatments main effects [i.e., two insecticide treatments (with and without), two alfalfa cultivars (Dominator, potato leafhopper-susceptible; WL358LH, potato leafhopper-tolerant), and three alfalfa-grass proportions (50:50, 75:25 and 100:0)] and their interactions for annual total forage and alfalfa yields and annual weeds mass, as well as total forage and alfalfa yields and weeds mass at the first, second and third harvests in the second post-seeding year (2023) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada.

Sainte-Anne-de-Bellevue 2021 in 2023																
	Annual				First harvest				Second harvest				Third harvest			
Treatment main effects	Total forage yield	Alfalfa yield	Grass yield	Weeds mass	Total forage yield	Alfalfa yield	Grass yield	Weeds mass	Total forage yield	Alfalfa yield	Grass yield	Weeds mass	Total forage yield	Alfalfa yield	Grass yield	Weeds mass
I†	0.3060	0.2661	0.6325	0.6080	0.3283	0.2925	0.4358	0.4957	0.2678	0.3141	0.3909	0.5339	0.3312	0.2343	0.2105	0.5880
C‡	0.2225	0.2017	0.1549	0.6848	0.6358	0.6037	0.1302	0.4832	0.0805	0.0864	0.8674	0.9279	0.1709	0.1836	0.2457	0.6093
P§	0.0701	0.0022	<0.0001	0.0948	0.0884	0.0004	<0.0001	0.1437	0.1083	0.0232	0.0002	0.2084	0.1922	0.0498	<0.0001	0.1941
I × C	0.8557	0.8395	0.0366	0.7050	0.2366	0.7422	0.0897	0.4883	0.8659	0.7521	0.3973	0.8663	0.8087	0.5642	0.0120	0.6177
I × P	0.3654	0.3286	0.2626	0.7860	0.1581	0.0679	0.4408	0.6908	0.8624	0.8446	0.0800	0.5326	0.5318	0.4791	0.0503	0.9137
C × P	0.3845	0.2970	0.1031	0.3173	0.7736	0.4366	0.0696	0.2981	0.3038	0.4143	0.9577	0.0496	0.1848	0.1276	0.0748	0.4801
I × C × P	0.3927	0.1485	0.1350	0.2879	0.4376	0.1006	0.2603	0.3619	0.2767	0.1793	0.2544	0.3377	0.7284	0.5187	0.0417	0.3294

† I, Insecticide treatments;
‡ C, Cultivars;
§ P, Alfalfa:grass proportions.

Table A4. P values of three treatments main effects [i.e., two insecticide treatments (with and without), two alfalfa cultivars (Dominator, potato leafhopper-susceptible; WL358LH, potato leafhopper-tolerant), and three alfalfa-grass proportions (50:50, 75:25 and 100:0)] and their interactions for annual total forage and alfalfa yields and annual weeds mass, as well as total forage and alfalfa yields and weeds mass at the first and second harvests in the seeding year (2022) at Sainte-Anne-de-Bellevue (SAB-B), QC, Canada.

Sainte-Anne-de-Bellevue 2022 in 2022												
Treatment main effects	Annual				First harvest				Second harvest			
	Total forage yield	Alfalfa yield	Grass yield	Weeds mass	Total forage yield	Alfalfa yield	Grass yield	Weeds mass	Total forage yield	Alfalfa yield	Grass yield	Weeds mass
I†	0.7335	0.6451	0.8934	0.9360	0.3988	0.8020	0.9963	0.2998	0.8949	0.5307	0.8272	0.0375
C‡	0.9861	0.1309	0.0700	0.6426	0.9392	0.1692	0.0330	0.8567	0.8892	0.3687	0.1564	0.5747
P§	0.6086	<0.0001	<0.0001	0.4926	0.0975	0.0434	<0.0001	0.3006	0.0016	<0.0001	<0.0001	0.0004
I × C	0.4311	0.4080	0.0622	0.9796	0.7268	0.2812	0.1116	0.8795	0.4685	0.8471	0.0694	0.7866
I × P	0.9361	0.8200	0.9434	0.2536	0.6307	0.1903	0.9899	0.8745	0.3225	0.8735	0.8002	0.0251
C × P	0.1319	0.0586	0.1361	0.1441	0.3146	0.1712	0.3812	0.1097	0.1164	0.0904	0.1027	0.3466
I × C × P	0.9707	0.4919	0.6611	0.7517	0.9601	0.2239	0.8954	0.6444	0.8366	0.7957	0.5227	0.6189

† I, Insecticide treatments;
‡ C, Cultivars;
§ P, Alfalfa:grass proportions.

Table A5. P values of three treatments main effects [i.e., two insecticide treatments (with and without), two alfalfa cultivars (Dominator, potato leafhopper-susceptible; WL358LH, potato leafhopper-tolerant), and three alfalfa-grass proportions (50:50, 75:25 and 100:0)] and their interactions for annual total forage and alfalfa yields and annual weeds mass, as well as total forage and alfalfa yields and weeds mass at the first, second and third harvests in the first post-seeding year (2023) at Sainte-Anne-de-Bellevue (), QC, Canada.

Sainte-Anne-de-Bellevue 2022 in 2023																
	Annual				First harvest				Second harvest				Third harvest			
Factors	Total forage yield	Alfalfa yield	Grass yield	Weeds mass	Total forage yield	Alfalfa yield	Grass yield	Weeds mass	Total forage yield	Alfalfa yield	Grass yield	Weeds mass	Total forage yield	Alfalfa yield	Grass yield	Weeds mass
I†	0.9268	0.8914	0.6398	0.8658	0.7175	0.7770	0.5858	0.9377	0.6144	0.6648	0.7854	0.5955	0.5444	0.6085	0.6939	0.9445
C‡	0.7225	0.7563	0.0009	0.6855	0.3834	0.7738	0.0071	0.8390	0.5077	0.3366	0.0581	0.4283	0.5825	0.7872	0.0784	0.4038
P§	0.7800	0.0020	<0.0001	0.0924	0.8969	<0.0001	<0.0001	0.0569	0.3167	0.0512	<0.0001	0.1733	0.9778	0.6482	<0.0001	0.3737
I × C	0.5286	0.9534	0.0044	0.2728	0.1795	0.5629	0.0438	0.4526	0.8169	0.7922	0.0089	0.1438	0.8983	0.7645	0.0516	0.7159
I × P	0.8790	0.7248	0.2864	0.7704	0.8803	0.4882	0.1484	0.8050	0.9429	0.9749	0.8802	0.7251	0.8993	0.9142	0.9211	0.5823
C × P	0.0746	0.3102	0.0044	0.8112	0.0866	0.3385	0.0055	0.3454	0.2906	0.4419	0.0677	0.4587	0.0858	0.2094	0.3113	0.5050
I × C × P	0.9260	0.8559	0.2321	0.3324	0.8927	0.5372	0.3273	0.4001	0.3565	0.6491	0.3424	0.4706	0.9657	0.9770	0.3092	0.5849

† I, Insecticide treatments;
‡ C, Cultivars;
§ P, Alfalfa:grass proportions.

Table A6. P values of three treatments main effects [i.e., two insecticide treatments (with and without), two alfalfa cultivars (Dominator, potato leafhopper-susceptible; WL358LH, potato leafhopper-tolerant), and three alfalfa-grass proportions (50:50, 75:25 and 100:0)] and their interactions for annual total forage and alfalfa yields and annual weeds mass in the seeding year (2021) at La Pocatière, QC, Canada.

La Pocatière 2021 in 2021				
	Annual			
Factors	Total forage yield	Alfalfa yield	Grass yield	Weeds mass
I†	0.4975	0.5070	0.5321	0.2873
C‡	0.2833	0.1218	0.1966	0.0658
P§	<0.0001	<0.0001	<0.0001	0.2212
I × C	0.6869	0.7261	0.1728	0.2135
I × P	0.5537	0.7002	0.6090	0.3246
C × P	0.5635	0.1611	0.5287	0.2956
I × C × P	0.4526	0.3667	0.4955	0.5489

† I, Insecticide treatments;
‡ C, Cultivars;
§ P, Alfalfa:grass proportions.

Table A7. P values of three treatments main effects [i.e., two insecticide treatments (with and without), two alfalfa cultivars (Dominator, potato leafhopper-susceptible; WL358LH, potato leafhopper-tolerant), and three alfalfa-grass proportions (50:50, 75:25 and 100:0)] and their interactions for annual total forage and alfalfa yields and annual weeds mass, as well as total forage and alfalfa yields and weeds mass at the first, second and third harvests in the first post-seeding year (2022) at La Pocatière, QC, Canada.

La Pocatière 2021 in 2022																
	Annual				First harvest				Second harvest				Third harvest			
Treatment main effects	Total forage yield	Alfalfa yield	Grass yield	Weeds mass	Total forage yield	Alfalfa yield	Grass yield	Weeds mass	Total forage yield	Alfalfa yield	Grass yield	Weeds mass	Total forage yield	Alfalfa yield	Grass yield	Weeds mass
I[†]	0.3820	0.5917	0.3212	0.6693	0.6765	0.9239	0.3535	0.9217	0.2043	0.1726	0.5777	0.4868	0.6352	0.7593	0.5595	0.7022
C[‡]	0.0023	0.0023	0.2779	0.6258	0.0101	0.0055	0.2599	0.8945	0.2094	0.1893	0.3411	0.9036	0.0106	0.0704	0.0167	0.3522
P[§]	0.0236	0.0464	<0.0001	0.2320	0.0020	0.0437	<0.0001	0.5447	0.7469	0.7334	<0.0001	0.6807	0.3395	<0.0001	0.1824	0.0006
I × C	0.1973	0.3083	0.5771	0.5573	0.2558	0.2491	0.7510	0.3564	0.1670	0.1251	0.1141	0.0758	0.0154	0.9818	0.0228	0.9653
I × P	0.4616	0.5699	0.7748	0.3953	0.3652	0.4615	0.7419	0.4125	0.2093	0.3305	0.0072	0.99088	0.6767	0.0377	0.7432	0.9261
C × P	0.2914	0.2163	0.6996	0.4190	0.1355	0.0432	0.5931	0.6164	0.4319	0.4033	0.4330	0.6470	0.6771	0.2445	0.7112	0.4545
I × C × P	0.7429	0.8083	0.9560	0.1623	0.7584	0.8202	0.9546	0.2079	0.8222	0.8264	0.1611	0.2236	0.1850	0.9392	0.3098	0.9045

[†] I, Insecticide treatments;
[‡] C, Cultivars;
[§] P, Alfalfa:grass proportions.

Table A8. P values of three treatments main effects [i.e., two insecticide treatments (with and without), two alfalfa cultivars (Dominator, potato leafhopper-susceptible; WL358LH, potato leafhopper-tolerant), and three alfalfa-grass proportions (50:50, 75:25 and 100:0)] and their interactions for annual total forage and alfalfa yields and annual weeds mass, as well as total forage and alfalfa yields and weeds mass at the first and second harvests in the second post-seeding year (2023) at La Pocatière, QC, Canada.

La Pocatière 2021 in 2023												
Treatment main effects	Annual				First harvest				Second harvest			
	Total forage yield	Alfalfa yield	Grass yield	Weeds mass	Total forage yield	Alfalfa yield	Grass yield	Weeds mass	Total forage yield	Alfalfa yield	Grass yield	Weeds mass
I[†]	0.5824	0.6293	0.5912	0.7722	0.3857	0.4165	0.6347	0.0511	0.2151	0.2477	0.9948	0.9889
C[‡]	0.0001	0.0002	0.2272	0.7467	0.0004	0.0008	0.1224	0.9463	0.0063	0.0040	0.7085	0.7314
P[§]	0.0796	0.3936	<0.0001	0.1039	0.0373	0.1436	<0.0001	0.6896	0.6122	0.7319	0.0174	0.0632
I × C	0.9898	0.8784	0.6295	0.5596	0.9993	0.8424	0.5127	0.5844	0.9800	0.9461	0.8497	0.4675
I × P	0.9465	0.9680	0.8850	0.9839	0.8111	0.8779	0.8318	0.6617	0.9382	0.9430	0.8985	0.9995
C × P	0.1540	0.1819	0.7222	0.0715	0.1178	0.1491	0.6597	0.6738	0.6618	0.6562	0.5550	0.0668
I × C × P	0.1555	0.1176	0.7834	0.7266	0.0856	0.0779	0.9350	0.3813	0.6745	0.7044	0.1045	0.8155

[†] I, Insecticide treatments;
[‡] C, Cultivars;
[§] P, Alfalfa:grass proportions.

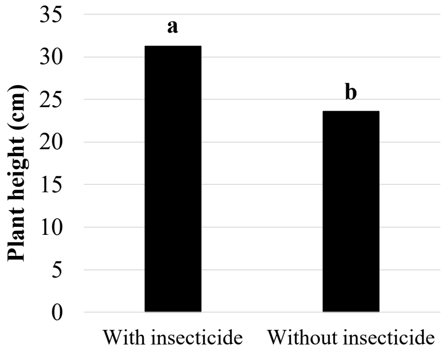

Figure A1. Alfalfa plant height at the first harvest in the seeding year at Sainte-Anne-de-Bellevue, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Two insecticide applications were done before the harvest. Values are the average of seven alfalfa cultivars.

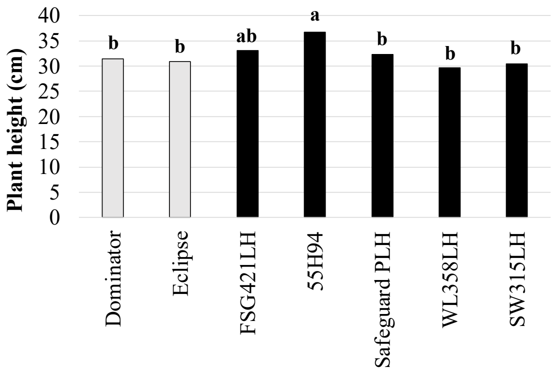

Figure A2. Alfalfa plant height at the second harvest in the seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at Sainte-Anne-de-Bellevue, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments (with and without).

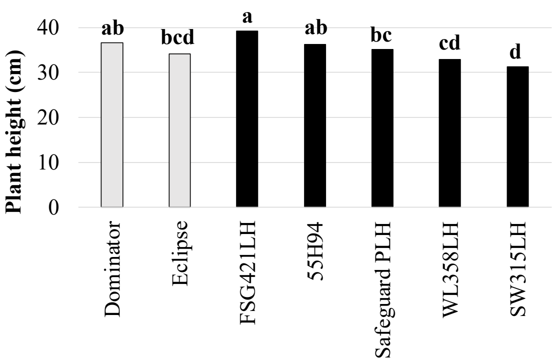

Figure A3. Plant height (cm) at the first harvest in the seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at La Pocatière, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments (with and without).

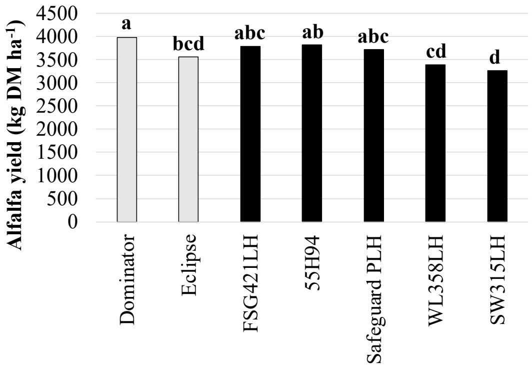

Figure A4. Alfalfa yield at the first harvest in the first post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at Sainte-Anne-de-Bellevue, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments (with and without).

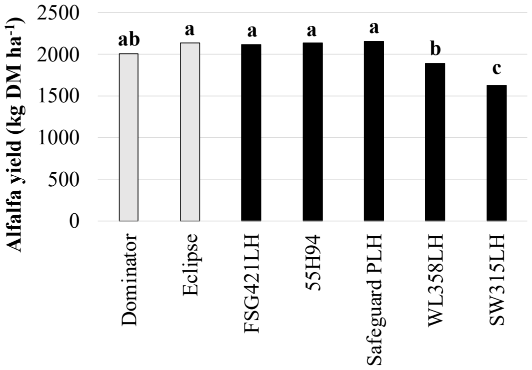

Figure A5. Alfalfa yield at the second harvest in the first post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at Sainte-Anne-de-Bellevue, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments (with and without).

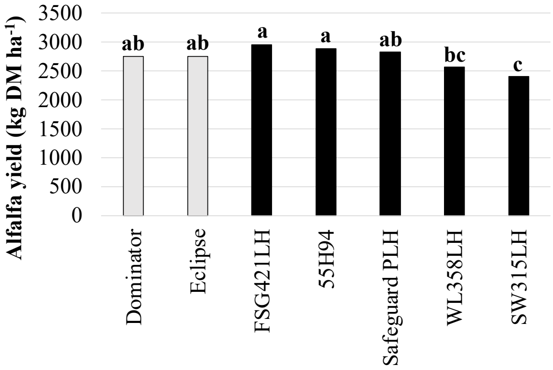

Figure A6. Alfalfa yield at the third harvest in the first post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at Sainte-Anne-de-Bellevue, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments (with and without).

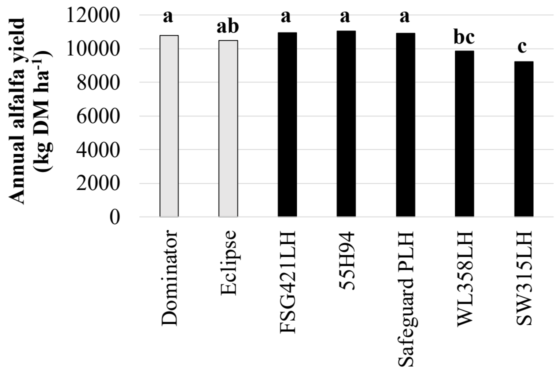

Figure A7. Annual alfalfa yield in the first post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at Sainte-Anne-de-Bellevue, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Values are the average of two insecticide treatments (with and without).

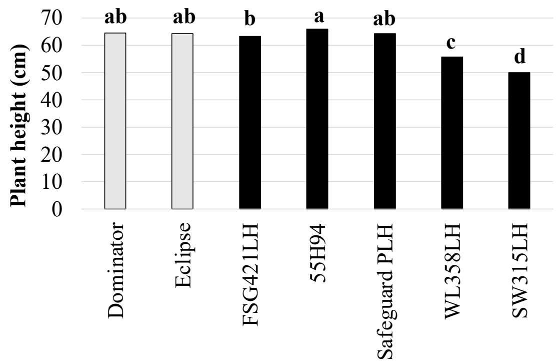

Figure A8. Alfalfa plant height at the second harvest in the first post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at Sainte-Anne-de-Bellevue, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Values are the average of two insecticide treatments (with and without).

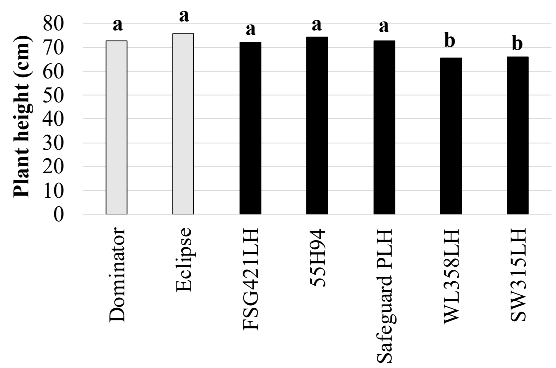

Figure A9. Alfalfa plant height at the third harvest in the first post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at Sainte-Anne-de-Bellevue, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Values are the average of two insecticide treatments (with and without).

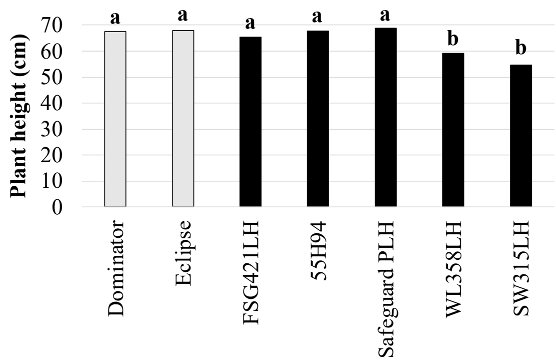

Figure A10. Alfalfa plant height at the fourth harvest in the first post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at Sainte-Anne-de-Bellevue, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments (with and without).

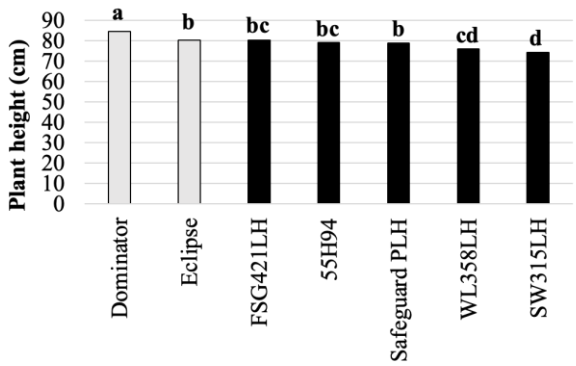

Figure A11. Alfalfa plant height at the second harvest in the first post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at La Pocatière, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments (with and without).

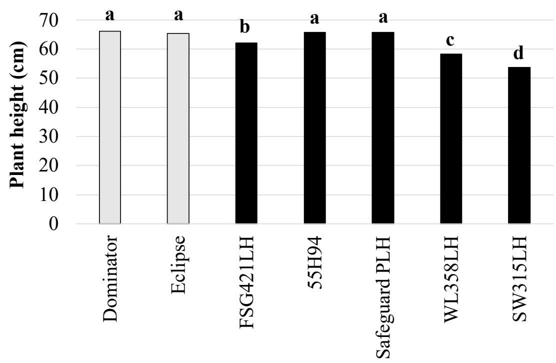

Figure A12. Alfalfa plant height at the third harvest in the first post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at La Pocatière, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments (with and without).

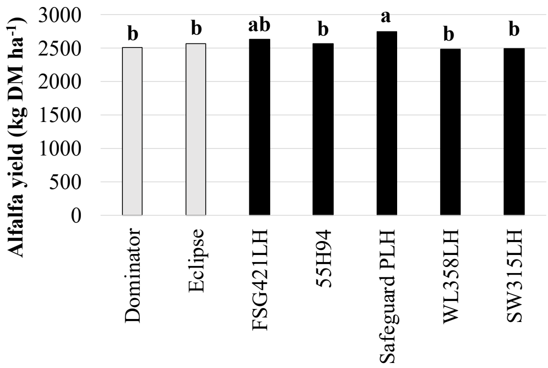

Figure A13. Alfalfa yield at the third harvest in the first post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at La Pocatière, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Values are the average of two insecticide treatments (with and without).

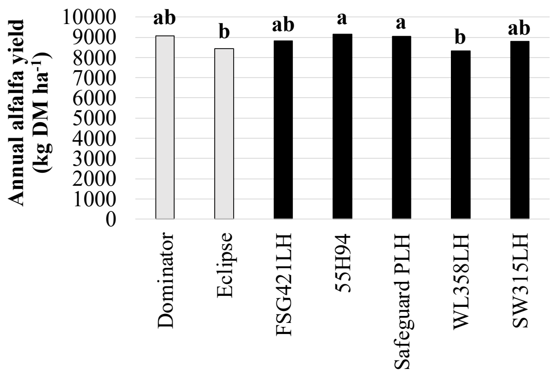

Figure A14. Annual alfalfa yield in the first post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at La Pocatière, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Values are the average of two insecticide treatments (with and without).

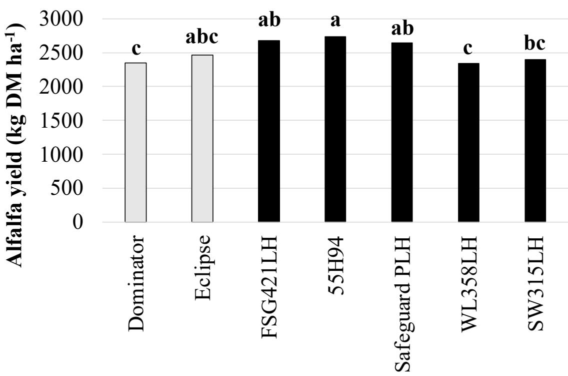

Figure A15. Alfalfa yield at the third harvest in the second post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at Sainte-Anne-de-Bellevue, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Values are the average of two insecticide treatments (with and without).

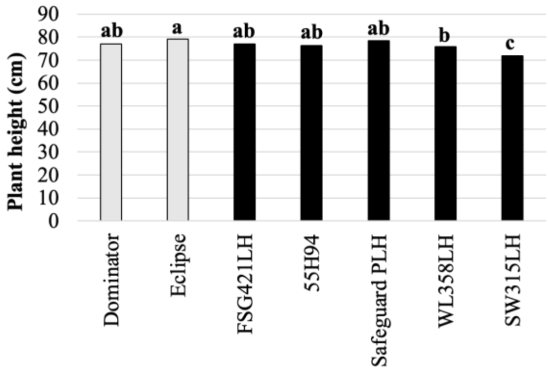

Figure A16. Alfalfa plant height at the first harvest in the second post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at Sainte-Anne-de-Bellevue, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Values are the average of two insecticide treatments (with and without).

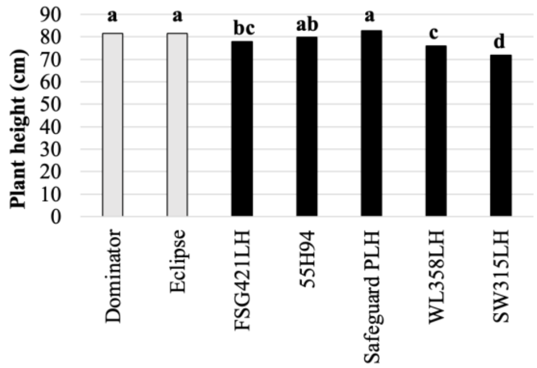

Figure A17. Alfalfa plant height at the second harvest in the second post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at Sainte-Anne-de-Bellevue, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Values are the average of two insecticide treatments (with and without).

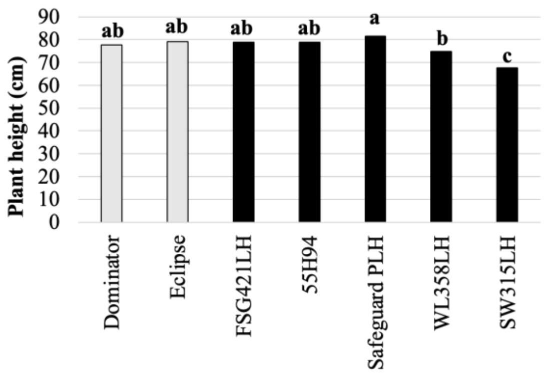

Figure A18. Alfalfa plant height at the third harvest in the second post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at Sainte-Anne-de-Bellevue, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Values are the average of two insecticide treatments (with and without).

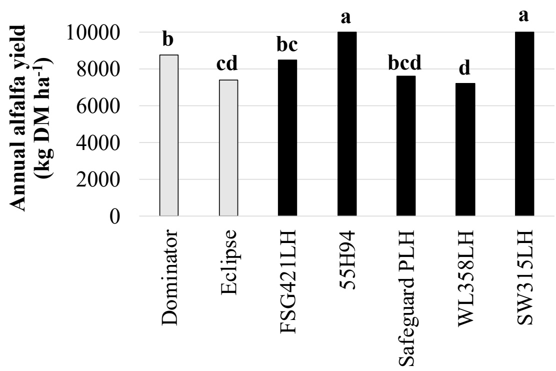

Figure A19. Annual alfalfa yield in the second post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at La Pocatière, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Values are the average of two insecticide treatments (with and without).

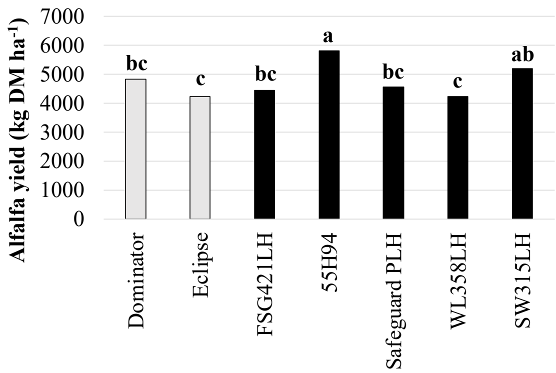

Figure A20. Alfalfa yield at the first harvest in the second post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at La Pocatière, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Values are the average of two insecticide treatments (with and without).

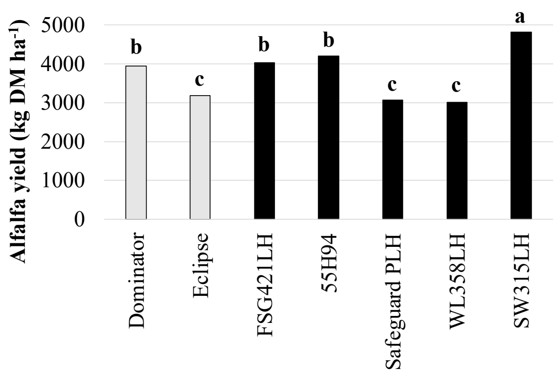

Figure A21. Alfalfa yield at the second harvest in the second post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at La Pocatière, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Values are the average of two insecticide treatments (with and without).

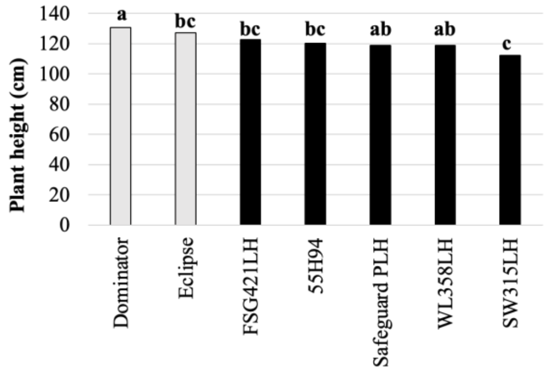

Figure A22. Alfalfa plant height at the first harvest in the second post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at La Pocatière, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Values are the average of two insecticide treatments (with and without).

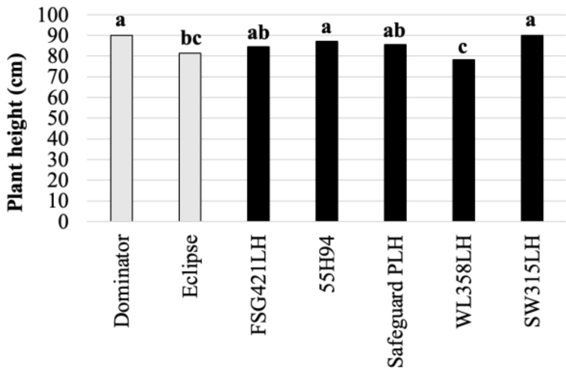

Figure A23. Alfalfa plant height at the second harvest in the second post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at La Pocatière, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Values are the average of two insecticide treatments (with and without).

Figure A24. Average acid detergent fiber (ADF) concentration in the seeding year at Sainte-Anne-de-Bellevue, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Three insecticide applications were done in the seeding year. Values are the average of seven alfalfa cultivars.

Figure A25. Average crude protein (CP) concentration in the seeding year at La Pocatière, QC, Canada. Means followed by different letters are significantly different (P < 0.05). One insecticide application was done in the seeding year. Values are the average of seven alfalfa cultivars.

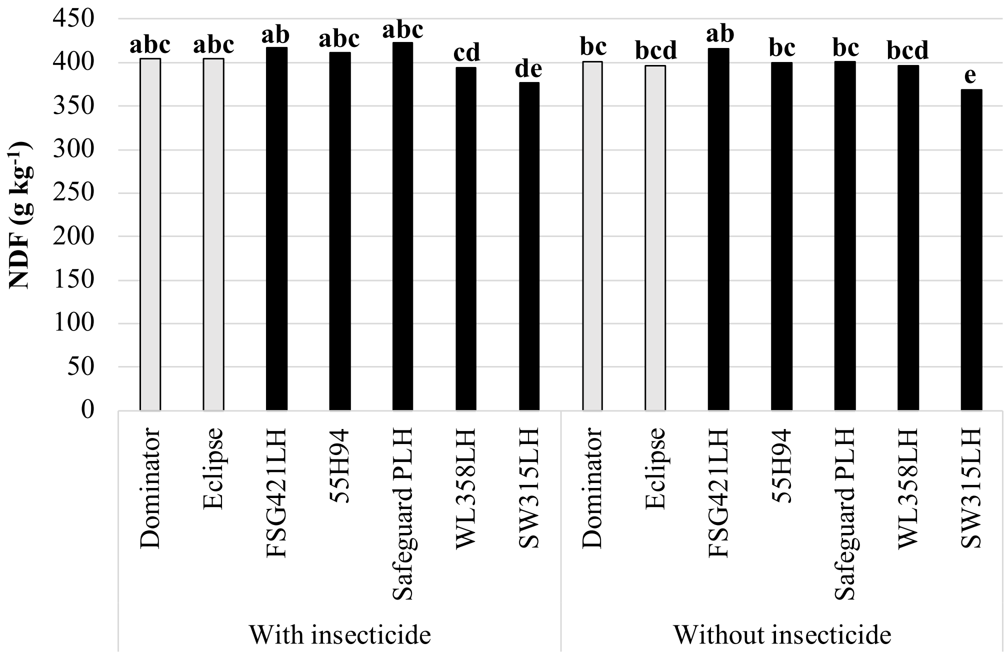

Figure A26. Average neutral detergent fiber (NDF) concentration in the second post-seeding year of five potato leafhopper-tolerant (black bars) and two potato leafhopper-susceptible (gray bars) alfalfa cultivars grown at Sainte-Anne-de-Bellevue, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Three insecticide applications were done in the seeding year.

Figure A27. Alfalfa plant height at the second harvest in the seeding year (2021) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada. Means followed by different letters are significantly different (P < 0.05). Three insecticide applications were done before the harvest. Values are the average of two alfalfa cultivars with three alfalfa-grass proportions.

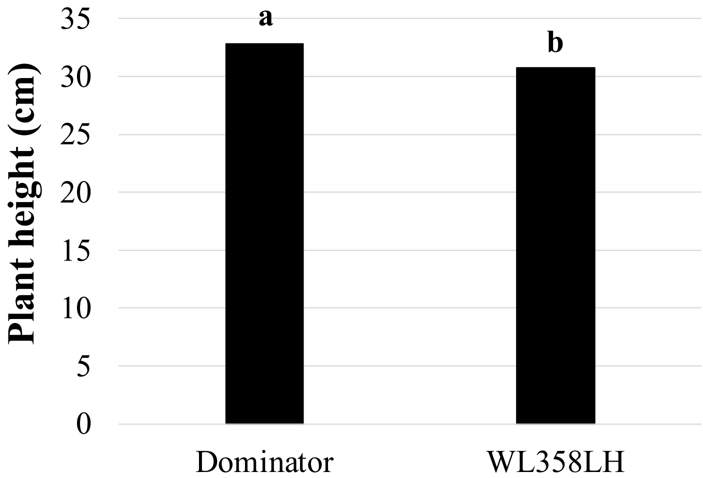

Figure A28. Alfalfa plant height at the first harvest in the seeding year (2021) at La Pocatière, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

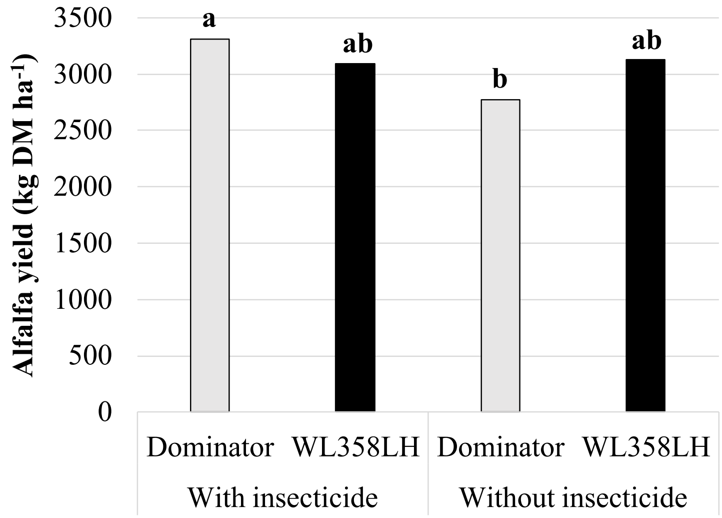

Figure A29. Alfalfa yield at the first harvest in the first post-seeding year (2022) of two alfalfa cultivars (Dominator, potato leafhopper susceptible; WL358LH, potato leafhopper-tolerant) submitted to two insecticide treatments (with and without) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Insecticide applications were done in the seeding year. Values are the average of three alfalfa-grass proportions (50:50, 75:25 and 100:0).

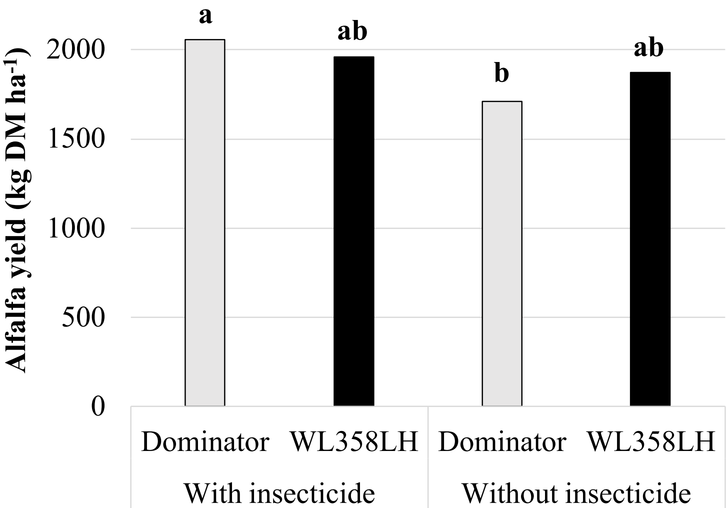

Figure A30. Alfalfa yield at the second harvest in the first post-seeding year (2022) of two alfalfa cultivars (Dominator, potato leafhopper susceptible; WL358LH, potato leafhopper-tolerant) submitted to two insecticide treatments (with and without) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Insecticide applications were done in the seeding year. Values are the average of three alfalfa-grass proportions (50:50, 75:25 and 100:0).

Figure A31. Alfalfa plant height at the first harvest in the first post-seeding year (2022) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

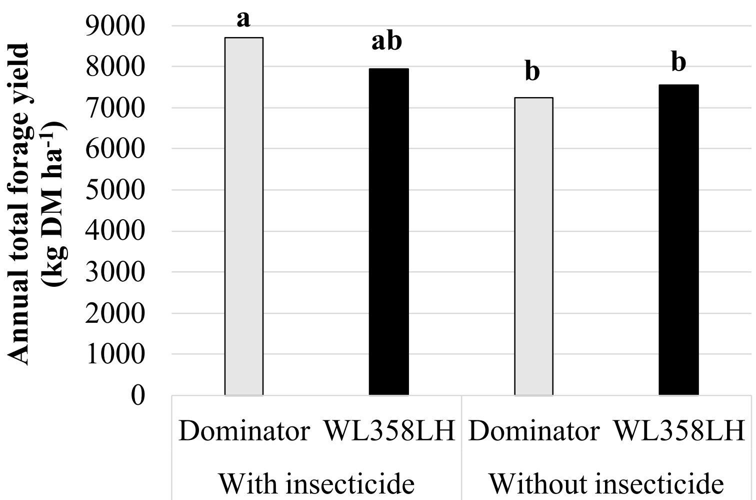

Figure A32. Annual total forage yield in the first post-seeding year (2022) of two alfalfa cultivars (Dominator, potato leafhopper susceptible; WL358LH, potato leafhopper-tolerant) submitted to two insecticide treatments (with and without) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada. Means followed by different letters are significantly different (P < 0.05). One insecticide application was done in the first post-seeding year. Values are the average of three alfalfa-grass proportions (50:50, 75:25 and 100:0).

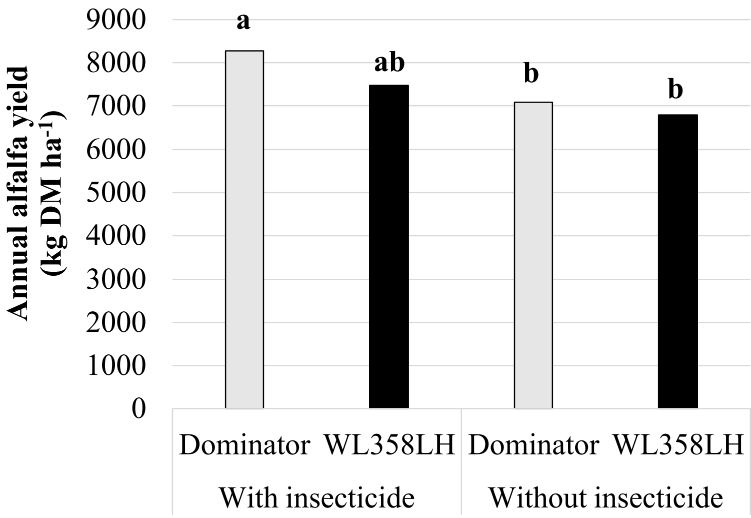

Figure A33. Annual alfalfa yield in the first post-seeding year (2022) of two alfalfa cultivars (Dominator, potato leafhopper susceptible; WL358LH, potato leafhopper-tolerant) submitted to two insecticide treatments (with and without) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). One insecticide application was done in the first post-seeding year. Values are the average of three alfalfa-grass proportions (50:50, 75:25 and 100:0).

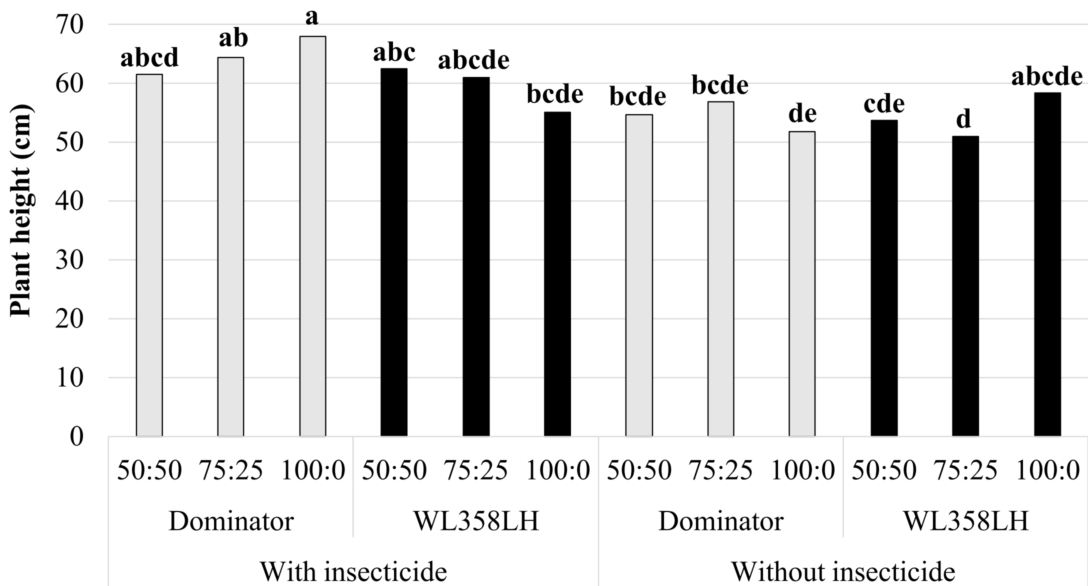

Figure A.34. Alfalfa plant height at the third harvest in the first post-seeding year (2022) of the combination of two alfalfa cultivars (Dominator, potato leafhopper-susceptible; WL358LH, potato leafhopper-tolerant), two insecticide treatments (with and without), and three alfalfa-grass proportions (50:50, 75:25 and 100:0) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Insecticide applications were done in the seeding year.

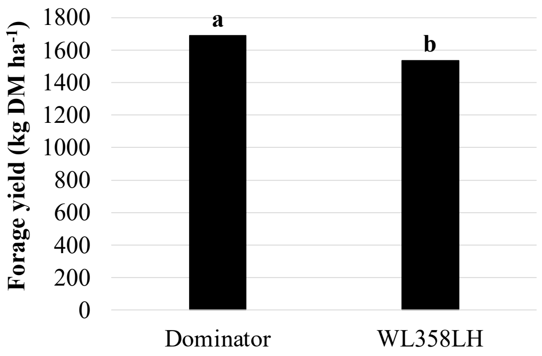

Figure A35. Total forage yield at the third harvest in the first post-seeding year (2022) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

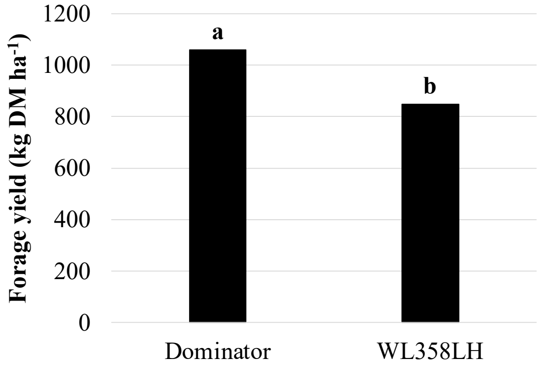

Figure A36. Total forage yield at the fourth harvest in the first post-seeding year (2022) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

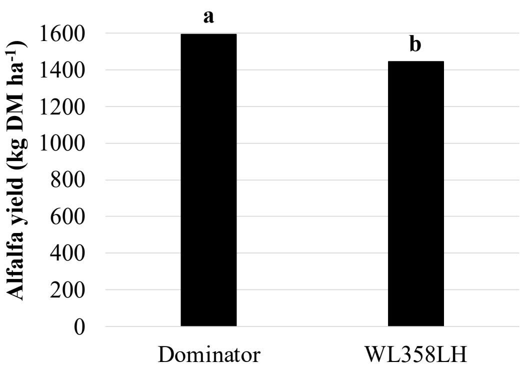

Figure A37. Alfalfa yield at the third harvest in the first post-seeding year (2022) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

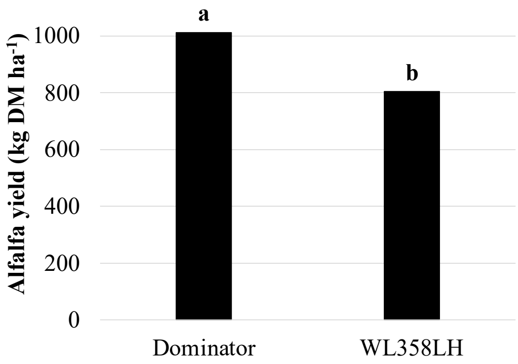

Figure A38. Alfalfa yield at the fourth harvest in the first post-seeding year (2022) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

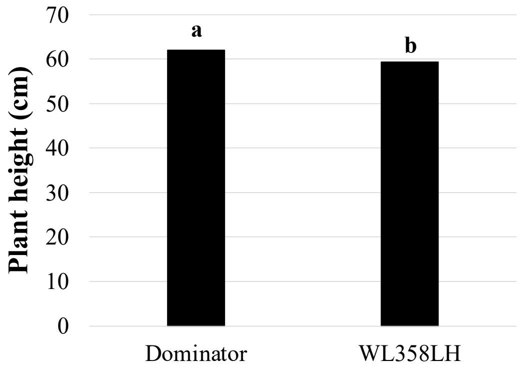

Figure A39. Alfalfa plant height at the second harvest in the first post-seeding year (2022) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

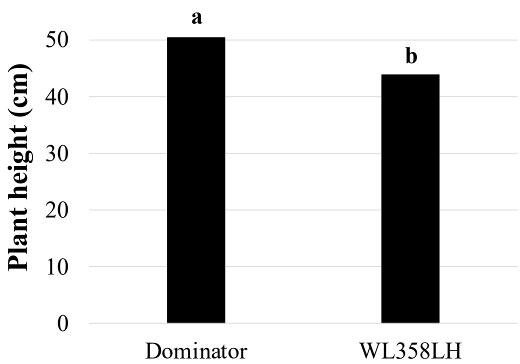

Figure A40. Alfalfa plant height at the fourth harvest in the first post-seeding year (2022) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

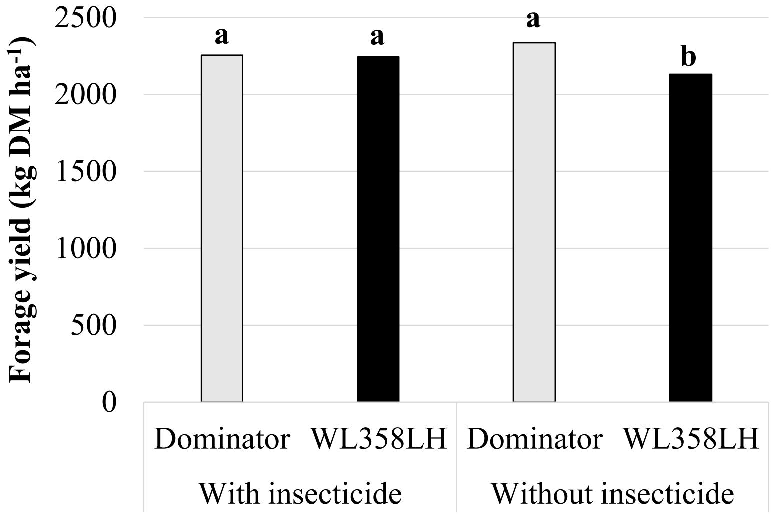

Figure A41. Total forage yield at the third harvest in the first post-seeding year (2022) of two alfalfa cultivars (Dominator, potato leafhopper susceptible; WL358LH, potato leafhopper-tolerant) submitted to two insecticide treatments (with and without) at La Pocatière, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Insecticide applications were done in the seeding year. Values are the average of three alfalfa-grass proportions (50:50, 75:25 and 100:0).

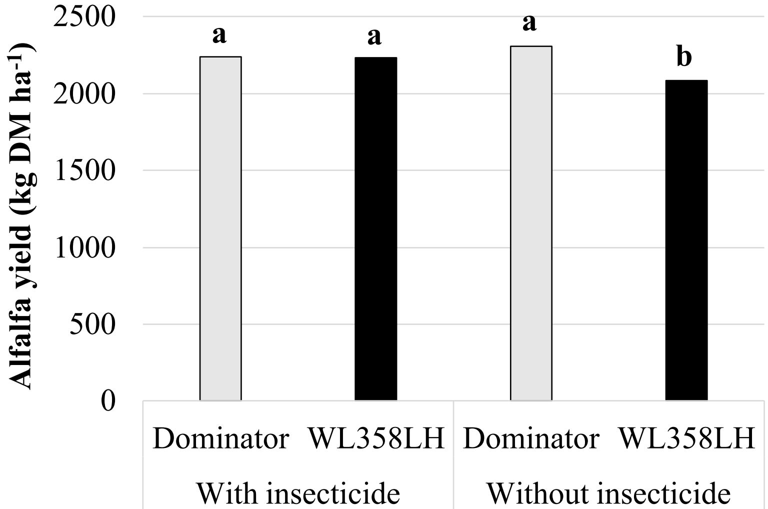

Figure A42. Alfalfa yield at the third harvest in the first post-seeding year (2022) of two alfalfa cultivars (Dominator, potato leafhopper susceptible; WL358LH, potato leafhopper-tolerant) submitted to two insecticide treatments (with and without) at La Pocatière, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Insecticide applications were done in the seeding year. Values are the average of three alfalfa-grass proportions (50:50, 75:25 and 100:0).

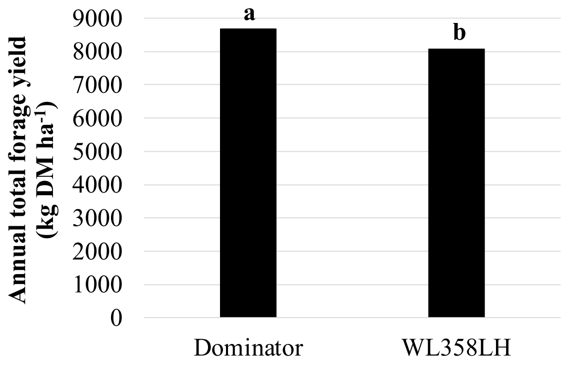

Figure A43. Annual total forage yield in the first post-seeding year (2022) at La Pocatière, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

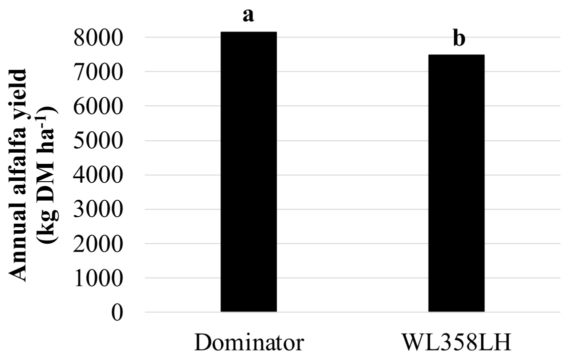

Figure A44. Annual alfalfa yield in the first post-seeding year (2022) at La Pocatière, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

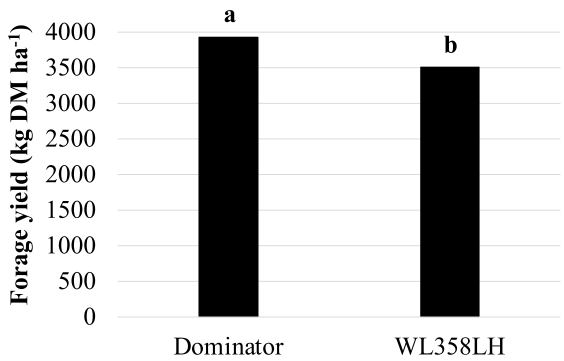

Figure A45. Total forage yield at the first harvest in the first post-seeding year (2022) at La Pocatière, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

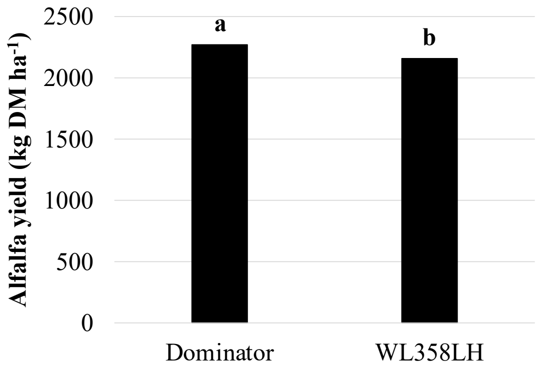

Figure A46. Total forage yield at the third harvest in the first post-seeding year (2022) at La Pocatière, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

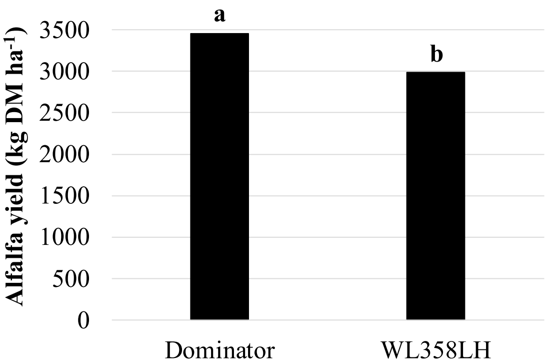

Figure A47. Alfalfa yield at the first harvest in the first post-seeding year (2022) at La Pocatière, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

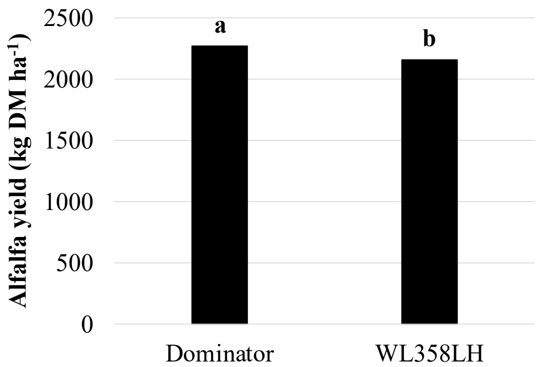

Figure A48. Alfalfa yield at the third harvest in the first post-seeding year (2022) at La Pocatière, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

Figure A49. Alfalfa plant height at the second harvest in the second post-seeding year (2023) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

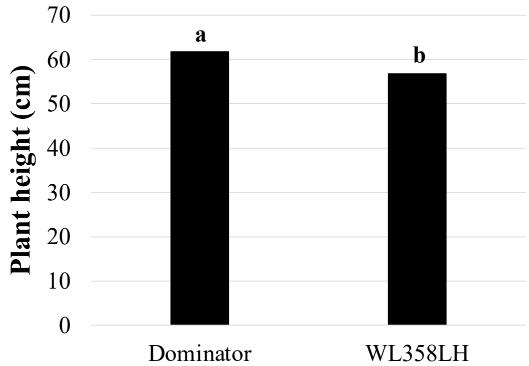

Figure A50. Alfalfa plant height at the first harvest in the second post-seeding year (2023) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

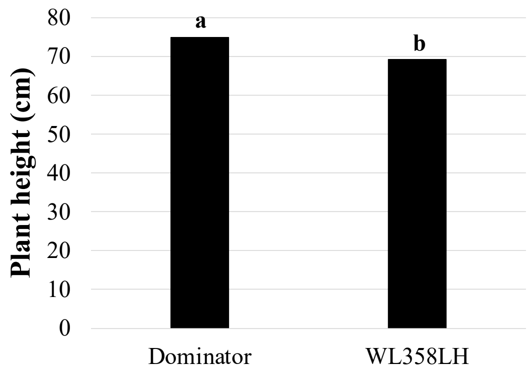

Figure A51. Alfalfa plant height at the third harvest in the second post-seeding year (2023) at Sainte-Anne-de-Bellevue (SAB-A), QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

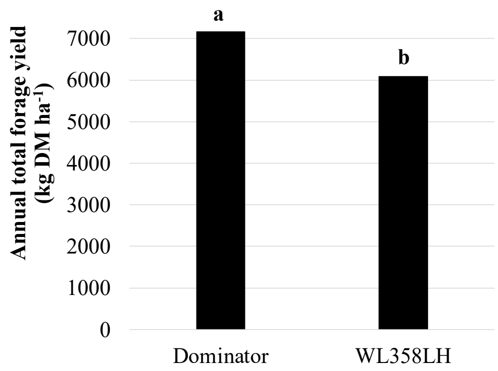

Figure A52. Annual total forage yield in the second post-seeding year (2023) at La Pocatière, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

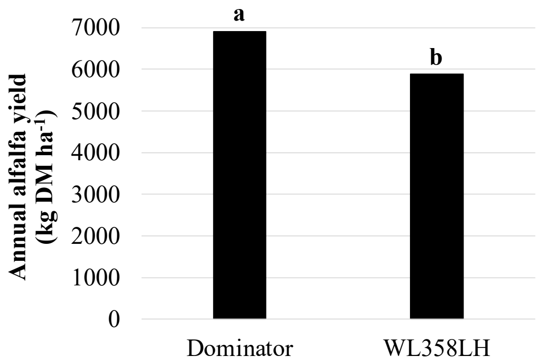

Figure A53. Annual alfalfa yield in the second post-seeding year (2023) at La Pocatière, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

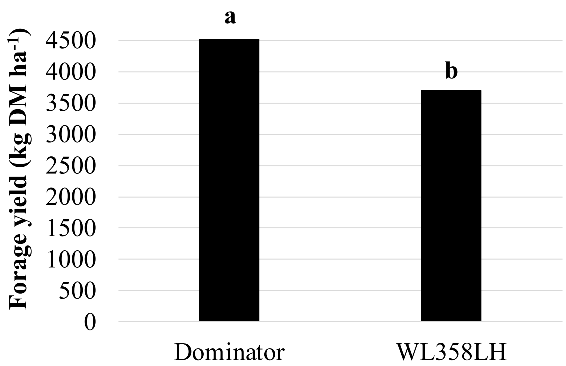

Figure A54. Total forage yield at the first harvest in the second post-seeding year (2023) at La Pocatière, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

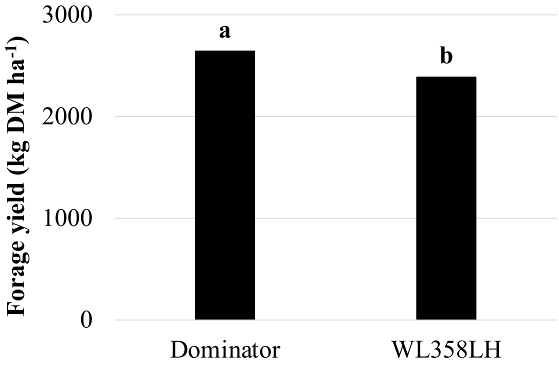

Figure A55. Total forage yield at the second harvest in the second post-seeding year (2023) at La Pocatière, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

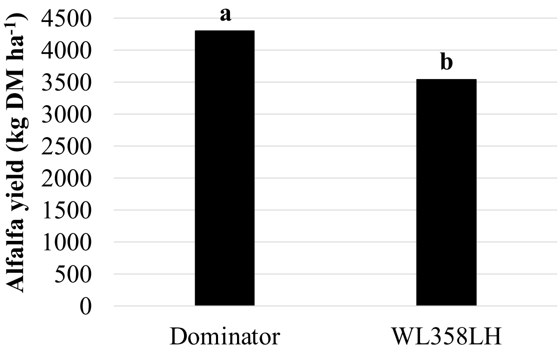

Figure A56. Alfalfa yield at the first harvest in the second post-seeding year (2023) at La Pocatière, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

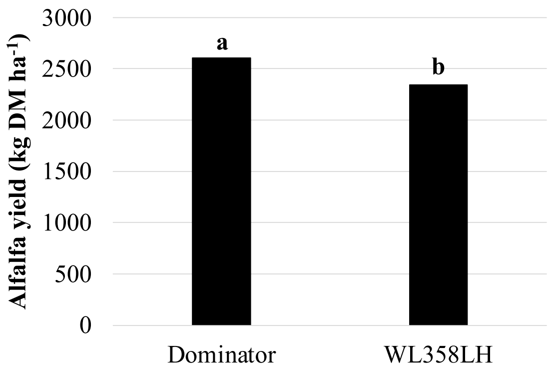

Figure A57. Alfalfa yield at the second harvest in the second post-seeding year (2023) at La Pocatière, QC, Canada. Means followed by different letters are significantly different (P < 0.05). Values are the average of two insecticide treatments with three alfalfa-grass proportions.

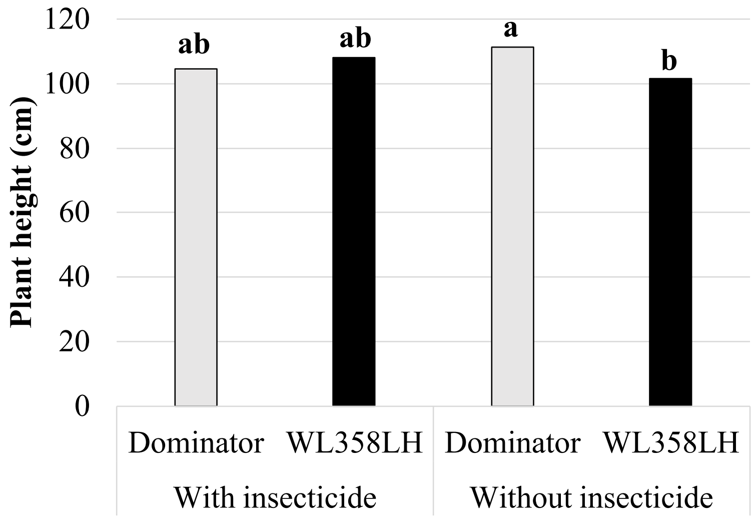

Figure A58. Alfalfa plant height at the first harvest in the second post-seeding year (2023) of two alfalfa cultivars (Dominator, potato leafhopper susceptible; WL358LH, potato leafhopper-tolerant) submitted to two insecticide treatments (with and without) at La Pocatière, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). One insecticide application was done in the seeding year. Values are the average of three alfalfa-grass proportions (50:50, 75:25 and 100:0).

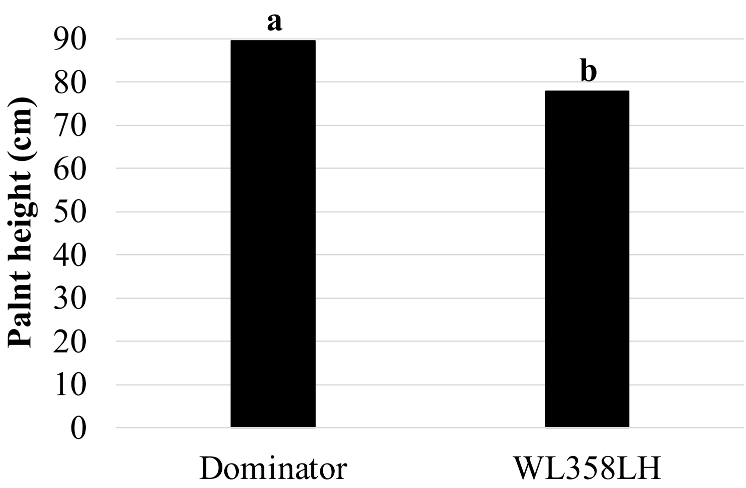

Figure A59. Alfalfa plant height at the second harvest in the second post-seeding year (2023) at La Pocatière, QC, Canada. Means followed by different letters are significantly different ($P < 0.05$). Values are the average of two insecticide treatments with three alfalfa-grass proportions.