THE EFFECT OF GROWTH REGULATORS AND NITROGEN ON FUSARIUM HEAD BLIGHT OF WHEAT

by

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Short Title

The Effect of Growth Regulators on Fusarium Head Blight of Wheat

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SHORT TITLE :

GROWTH REGULATORS AND NITROGEN

IN RELATION TO SCAB OF WHEAT

Mohamad Taufik Fauzi©

DEDIKASI

kepada istriku Nilhamdiah

kepada anak-anakku

Prastudy Mungkas Fa.. i dan Tuning Ridha Addiny

yang dengan rela berkorban dan memberikanku

kekuatan moral dan inspirasi dalam belajar

FOREWORD

This thesis consists of five parts. The first part is a general introduction and literature review presenting the problem, the goals of this research, and the theory and previous knowledge on the thesis topic. Parts two, three, and four are the body of this thesis presented as complete manuscripts covering the entire research project. Part five is a general discussion and conclusions of the whole manuscripts.

The thesis format has been approved by the Faculty of Graduate Studies and Research of McGill University and follows the condition outlined in the "Guideline Concerning Thesis Preparation", section B.2, **"Manuscripts and Authorship"**, which are as follows:

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All the work reported here was the responsibility of the candidate. The research was conducted under the supervision of Dr. T.C. Paulitz, Department of Plant Science, Macdonald Campus of McGill University. For consistency and convenience, all manuscripts follow the same format. The copies that will be sent to respective journals, however, will follow the requirements of each journal. All manuscripts are co-authored by M.T. Fauzi and T.C. Paulitz.

ABSTRACT

M. Sc. M. T. Fauzi Plant Science

THE EFFECT OF GROWTH REGULATORS AND NITROGEN ON FUSARIUM HEAD BLIGHT OF WHEAT

Plant growth regulators and nitrogen fertilization have been associated with the increased incidence of fusarium head blight, a destructive disease of wheat (*Triticum aestivum* L.). In Canada, the major causal organism of this disease is *Fusarium graminearum* Schwabe, the conidial state of *Gibberella zeae* (Schw.) Petch. Most studies concerning the effect of plant growth regulators on fusarium head blight were conducted in fields with natural infection. The objective of this research was to evaluate the effect of growth regulators and nitrogen fertilizer on the incidence of fusarium head blight of wheat with artificial inoculations.

A survey conducted in a field trial testing the effect of the plant growth regulator Cerone on the yield components of several cultivars of spring wheat showed that Cerone treatments increased *Fusarium* infection only in cultivar Columbus. Further research was conducted using cultivar Max, a cultivar susceptible to fusarium head blight, which is widely grown in Quebec. In controlled-condition greenhouse trials, the growth regulators Cycocel and Cerone, as well as nitrogen fertilization did not influence the disease progress. In the 1991 field experiment, the highest incidence of seed

infection was observed in Cycocel treatments when the macroconidia of *F. graminearum* were directly applied to the heads, but not significantly different from the non-treated control. None of the nitrogen levels affect the incidence of seed infection. In the 1992 field trial, the plots were treated with macroconidia of *F. graminearum* applied to the heads or with *Fusarium*-colonized corn applied to the rows. Both Cycocel and Cerone significantly increased the incidence of spikelet only in the colonized corn treatments. Cycocel also increased the incidence of seed infection, but only in colonized corn treatments. Cycocel also increased the incidence of seed infection in the non-inoculated treatments. Growth regulators had no effect on the disease when heads were inoculated directly with macroconidia.

RÉSUMÉ

M.Sc. M. T. Fauzi Plant Science

EFFET DE RÉGULATEURS DE CROISSANCE ET DE L'AZOTE SUR LA FUSARIOSE DU BLÉ

L'augmentation de l'incidence de la fusariose, sévère maladie du blé (*Triticum aestivum* L.), a été associée aux apports de régulateurs de croissance et de fertilisants azotés. Au Canada, le principal champignon responsable de la maladie est *Fusarium graminearum* Schwabe, génération asexuée de *Gibberella zeae* (Schw.) Petch. La majorité des travaux portant sur l'effet des régulateurs de croissance sur la fusariose ont été réalisés dans des champs naturellement infestés. L'objectif de recherche est de déterminer l'effet de ces apports sur l'incidence de la fusariose du blé en ayant recourt à des inoculations artificielles pour les expériences en serre et en champs.

Lors d'une étude visant à déterminer l'effet d'ajout d'un régulateur de croissance (cerone) sur les rendements de plusieurs cultivars de blé de printemps, des observations ont montré que les traitements de cerone augmentaient l'infection par *Fusarium* seulement pour le cultivar Columbus. Des études subséquentes ont été menées sur un cultivar susceptible à la fusariose, le cultivar Max. Lors d'essais en serre dans des conditions contrôlées, les résultats ont démontrés que les

régulateurs de croissance cycocel et cerone ainsi que la fertilisation en azote n'influencent pas la progression de la maladie. En 1991 lors d'essais au champs, la plus forte incidence d'infection de graines fut observée dans les traitements de cycocel où les macroconidies de *F. graminearum* avaient été appliquées directement sur l'épi. Toutefois, les résultats n'étaient pas significativement différents de ceux obtenus chez le témoins non-traités. L'application d'azote n'a pas eu d'effet notable sur l'incidence d'infection des graines. En 1992, l'inoculation des parcelles a été réalisée avec soit des macroconidies de *F. graminearum* ou par épandage sur les rangs des grains de maïs colonisés avec *Fusarium*, l'inoculum était alors composé d'ascospores expulsées des périthèces produits sur les grains de maïs. Tant le cycocel que le cerone ont augmenté l'incidence d'infection des épillets mais ce, seulement chez les traitements utilisant le maïs colonisé comme source d'inoculum. Cependant pour ce même type d'inoculum, seul le cycocel a accru l'incidence de l'infection des grains de blé. Ce dernier a aussi a accru l'infection des grains de blé dans les traitements non-inoculés. Les régulateurs de croissance n'ont pas influencé la maladie lorsque les épis avaient été inoculés avec les macroconidies.

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I. GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1. GENERAL INTRODUCTION

Among agricultural products, wheat holds an important role in the Canada economy because 75 percent of the wheat produced is exported, and it supplied approximately 20 percent of the world market. Moreover, Canadian wheat is famous for its high quality and protein (Brigle and Curtis, 1987; Canada Yearbook 1990), so that it is preferred and highly valued by consumers. Efforts have been made in Quebec to increase the yield and to improve the quality of wheat. There has been interest in adopting intensive cereal management systems, which are widely used in Europe. Heavy nitrogen fertilization is an important part of this management strategy.

Nitrogen applications have been reported to be associated with increases in yield. Feyerherm *et al.* (1988) observed up to 25 percent increases in yield when nitrogen fertilizers were applied. The heavy use of nitrogen, however, can promote lodging, which can reduce potential yield. Therefore, the use of plant growth regulators as 'anti-lodging agents' will be very important to maintain the yield (Dahnous *et al.*, 1982; Pearson *et al.*, 1989). Many authors reported that plant growth regulators such as chlormequat chloride and ethephon have been

used successfully to reduce lodging on cereals (Dahnous *et al.*, 1982; Pearson *et al.*, 1989).

An awareness of the presence of iatrogenic plant diseases, diseases which are enhanced by chemical applications, encouraged researchers to study the influence of such management on the severity of plant diseases. Those agrochemicals may affect the host plant, the pathogen, or the ecosystem (Griffiths, 1981).

One of the important diseases in wheat is fusarium head blight (scab), which can constrain the yield up to 50 percent and cause inferior quality and low grade of wheat (Jones and Clifford, 1983; Sutton, 1982). Growing concern has been given to the disease since the *Fusarium* species associated with it produce mycotoxins that are hazardous to humans and livestock (Sutton, 1982).

The disease in Canada is caused mainly by *Fusarium graminearum* (Schwabe), the conidial state of *Gibberella zeae* (Schw.) Petch (Sutton, 1982). Other fusaria such as *F. culmorum, F. avenaceum, F. sporotrichioides, F. poae, and F. equiseti* have also been found to be associated with fusarium head blight in Canada (Duthie *et al.*, 1986), but they are generally less important than *F. graminearum* (Cunfer, 1987).

Results of the studies of the effect of nitrogen application on plant diseases have been controversial, while the publications on the effect of plant growth regulators on plant diseases are rare. Most studies concerning the plant growth regulator effect on fusarium head blight were conducted in the field with natural infection.

The objective of this work was to determine the influence of applying plant growth regulators and nitrogen fertilizer on fusarium head blight incidence of spring wheat. To meet this objective, work was carried out using artificial inoculation and imitation of natural inoculation. This thesis consists of three parts. The first part was a survey of *Fusarium* spp. in the kernels of spring wheat and the effect of a plant growth regulator on *Fusarium* infection of several cultivars of wheat in a field trial at Macdonald Campus of McGill University in 1990. The second part consisted of a study of the influence of plant growth regulator and nitrogen fertilizer applications on fusarium head blight incidence under greenhouse conditions. The third part involved two field studies using different methods of artificial inoculation.

1.2. LITERATURE REVIEWS

1.2.1. The crop : Common wheat (*Triticum aestivum* L.em.Thell)

1.2.1.1. The importance

Wheat is the number one food grain consumed by humans. It also can be used for feeding livestock when the price is not too high compared with other coarse grains such as corn. Low-grade and damaged grains can also be used in livestock feeding. They can also be fermented for alcohol production and can be used in the starch industry (Brigle and Curtis, 1987).

In Canada, wheat is the major exported grain. More than half of all grain exported in 1991 was wheat (Canada Grains Council, 1991). The value of exported wheat in 1992 (up to May 1992) exceeded $ 322 million (Statistics Canada, 1992).

1.2.1.2. Wheat management

Nitrogen fertilizer. The main purpose of improving the wheat quality is to increase the nutritional content, primarily protein. Nitrogen application as an important part of wheat management has been reported to be associated with high protein content as well as high yield. Halvorson *et al.* (1987)

reported that the application of nitrogen had a direct effect on the protein content of grain, and showed that adding more nitrogen can increase the protein of grain. Bruckner and Morey (1988) found that nitrogen fertilizer increased the yield of soft red winter wheat. Cox *et al.* (1989) and Bruckner and Morey (1988) found similar increases in yield in response to nitrogen fertilization. However, a negative correlation between the protein content and the yield has also been reported (Bajaj, 1990). Before applying nitrogen, it is important to know the nitrogen content of the soil for better prediction of nitrogen fertilizer requirements. The method and time of nitrogen application can affect the grain yield and the grain protein (Halvorson *et al.*, 1987).

Plant growth regulator application. The use of plant growth regulators became an important part of wheat management since the heavy use of nitrogen fertilizer can promote plant lodging. Lodging is a very serious problem in wheat production. Humphries (1968) stated that lodging can constrain the yield up to 50 percent. The lodging intensity can be higher with the higher amount of nitrogen application, since nitrogen increases plant succulence. The major cause of increased lodging due to N fertilizer additions is increased plant height, coupled with heavier head on top. CCC [(2 chloroethyl) trimethylammonium chloride = chlormequat] with Cycocel as the trade name has been reported to reduce and

prevent lodging (Humphries, 1968). Another plant growth regulator that has been reported to prevent lodging is ethephon [(2-chloethyl) phosphonic acid or Cerone as the trade name] (Dahnous *et al.*, 1982). These growth regulators reduce or prevent lodging through their ability to shorten the plant and strengthen the stem (Dahnous *et al.*, 1982; Pearson *et al.*, 1989).

1.2.2. The disease : Fusarium head blight

1.2.2.1. The causal organism

The causal agent of fusarium head blight is *Fusarium graminearum* (Schwabe)(= *F. roseum* Link. emend. Snyd. & Hans. 'Graminearum'), the conidial state of *Gibberella zeae* (Schw.) Petch. (Cook, 1981; Sutton, 1982).

There are two groups of *F. graminearum*, i.e. : (1) group I, which has not been reported to form perithecia, is associated with crown rot of wheat, and (2) group II, which readily forms perithecia on host materials or residues, and is the causal organism of fusarium head blight or scab (Cook, 1987; Francis and Burgess, 1977).

1.2.2.2. The description of the organism

The asexual state, *F. graminearum*, grows rapidly on PDA culture with yellow to tan aerial mycelium, margins white to carmine red, and with carmine red on the undersurface. The abundant macroconidia, which are sickle-shaped to almost straight with distinct foot-cells, are formed in sporodochia. Macroconidia are relatively large (20 - 70 μm) , while microconidia are absent (Jones and Clifford, 1983; Nelson *et al.*, 1983; Nirenberg, 1981).

The perfect state, *G. zeae*, forms black, ovoid perithecia with very rough tuberculate walls in host materials or residues. Asci are clavate with a short stipe and usually contain 8 ascospores. Ascospores, which are straight or curved, are usually 3-septate, hyaline to very light brown (Booth, 1971; Jones and Clifford, 1983).

1.2.2.3. Symptoms and disease cycle

The symptoms of fusarium head blight can be recognized easily from the brownish colour of immature spikelets as a result of premature death or blighting. Sometimes the fungus forms small dark perithecia and superficial mycelia and sporodochia that give a pinkish colour on spikes (Prescott *et al.*, 1986; Wiese, 1977).

The main inoculum source of the fusarium head blight pathogen is host debris on the soil. The pathogen survives as mycelia, conidia, ascospores or perithecia and multiplies in infected cereal residues in soil (Sutton, 1982; Wiese, 1977). Ascospores are discharged from perithecia and are mainly dispersed by wind, while macroconidia are dispersed by splashing or wind-driven rain (Sutton, 1982). Wiese (1977) stated that infections usually occur at anthesis.

1.2.2.4. The importance

Fusarium head blight can cause wheat to produce inferior quality of grain that is white (chalky) and shrivelled (Teich, 1989; Tuite *et al.*, 1990). It reduces yield as much as 50 percent (Jones and Clifford, 1983; Maric, 1981).

The growing importance of the disease has been due to the production of mycotoxins in infected kernels. There are two main mycotoxins produced by the organism. Firstly, deoxynivalenol (DON), also known as vomitoxin, can cause skin lesions, vomiting, and diarrhea to humans and livestock (Sydenham *et al.*, 1989). A DON concentration of more than 2 μg/g grain is unfit for food or feed (Teich, 1989). The second toxin, zearalenone can cause reproductive problems in certain animals, especially swine (Sydenham *et al.*, 1989).

Concentrations of zearalenone from 1 to 5 μg/g can cause estrogenism in swine (Sutton, 1982).

1.2.3. Wheat management and the disease

There are controversial results on the studies of nitrogen application effect on plant disease. Huber and Watson (1974) reported that the form of nitrogen affects disease severity, not the amount. Teich (1987) also found that fusarium head blight incidence was lower when the wheat was fertilized with urea rather than ammonium nitrate. Other authors such as Cook (1981) and Maric (1981) have stated that higher amounts of nitrogen applied can increase the disease severity of crown rot, foot rot, and head blight caused by *Fusarium* species.

The publications on the effect of plant growth regulators on plant diseases are rare. However, more attention has recently been given to this subject (Reddy and Strzelczyk, 1989). Since plant growth regulators can act like herbicides, the effects of herbicides on plant diseases are also reviewed.

Some authors reported that the use of plant growth regulators and herbicides tends to increase plant diseases (Griffiths, 1981). Graham and Linderman (1981) found that root

infection of Douglas-fir by *Fusarium* increased two fold when ethephon was applied to the plant. In vitro studies by Michniewicz and Czerwinska (1991), however, showed that ethephon inhibited the growth of *Fusarium culmorum, Alternaria tenuis* (*A. alternata*), *Cladosporium lignicolum*, and *Trichoderma lignorum*. These findings may lead to a hypothesis that the effects of plant growth regulators on the plants or hosts have an important role on the disease outcome, especially in fusarium head blight of wheat since plant growth regulators are applied before infection takes place.

The mechanisms involved in the disease increase after applying plant growth regulators and herbicides on the plants are changes in host composition and structure, metabolite leakage to the host surface, and changes in natural defense mechanisms (Griffiths, 1981).

Griffiths (1981) reported that some herbicides change the sugar concentration of host tissues, which in turn can affect pathogens that prefer tissues with either low or high sugar contents. The changes in plant structure which will lead to increasing diseases usually occur when plant growth regulators and herbicides are applied. Bockmann (1968) and Jones and Clifford (1983) reported that CCC increased fusarium head blight incidence due to the resulting dwarf habit.

The increase in leakage of metabolites to the plant surface after herbicide applications can lead to the increase of plant diseases (Griffiths, 1981). These metabolites may be used as nutrients by the pathogens for their development.

Altman and Campbell (1977) stated that herbicides predisposed sugar beets to infection by *Rhizoctonia solani*. This predisposition may occur as herbicides inhibit the production of some substances, such as phytoalexins, that are important for self defence of the plant (Levesque and Rahe, 1992).

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PREFACE TO CHAPTER 2

The experiments reported in Chapter 2 were conducted to determine which *Fusarium* species infected wheat seeds at a site in Quebec and to see the effect of the plant growth regulator Cerone on *Fusarium* infection of several cultivars of spring wheat. Results were used for further studies on the effect of growth regulators on the incidence of fusarium head blight. Each table and figure is presented on the page following the first referral to it.

II. NATURAL INFECTION OF SPRING WHEAT CULTIVARS BY *FUSARIUM* SPP. AND THE EFFECT OF A PLANT GROWTH REGULATOR

2.1. ABSTRACT

Wheat seeds (*Triticum aestivum* L.) from a field experiment testing the effect of the plant growth regulator Cerone on the yield of several cultivars of spring wheat were surveyed to determine the natural infection by *Fusarium* species. Seed infection was analyzed by plating out the seeds on 'half-strength PDA+PCNB' medium. The four most predominant species that were found in this survey were *Fusarium graminearum, F. sporotrichioides, F. poae,* and *F. equiseti*. The level of *Fusarium* infection in machine-harvested seeds was significantly higher in the cultivars Columbus and Max, compared to in Messier and Katepwa. A significant difference in infection between the cerone treatment and the control treatments was seen only in cultivar Columbus, in which 11% of seeds were infected with *Fusarium* spp. in the Cerone treatment, but only 3% in the control treatment. There were no significant differences in seed infection by *Fusarium graminearum* among the treatments. In hand harvested seed, infection of cultivar Columbus was significantly higher than in the other cultivars, but Cerone had no effect on *Fusarium* infection.

2.2. INTRODUCTION

In Quebec, there has been interest in adopting Intensive Cereal Management (ICM) practices, which have been used successfully to increase wheat yields in Europe. One of the important strategies in this practice is the use of high levels of nitrogen fertilizer. Heavy nitrogen fertilization, however, has been reported to be associated with increasing lodging intensity (Pumphrey and Rubenthaler, 1983).

Lodging is the major limitation in maximizing the yields of wheat (Wiersma *et al.*, 1986). Lodging can reduce wheat yield as much as 70 percent, depending on weather conditions (Linser, 1967).

Wiersma *et al.* (1986) stated that lodging can be reduced by the strict use of nitrogen fertilization and using dwarf, lodging-resistant cultivars. Another method that showed promising results in preventing lodging was using plant growth regulators such as chlormequat chloride (trade name = Cycocel) and ethephon (trade name = Cerone) (Dahnous *et al.*, 1982; Humphries, 1968).

These antilodging methods, however, have some disadvantages. Using less nitrogen fertilizer will lead to limitation of yields and protein content of grain (Bruckner

and Morey, 1988; Halvorson *et al.*, 1987). Mesterhazy (1991) reported that plant height is a morphological trait that affects natural infection of wheat scab, so that dwarf cultivars may be more susceptible to the disease. Plant growth regulators have been reported to increase disease severity, especially fusarium head blight (Bockmann, 1968; Martin *et al.*, 1991).

The main causal agent of fusarium head blight in Canada is *Fusarium graminearum* (Schwabe), the anamorph of *Gibberella zeae* (Schw.) Petch (Sutton, 1982). Duthie *et al.* (1986) have reported other *Fusarium* spp. that infect winter wheat seeds in eastern Canada were *F. sporotrichioides, F. avenaceum, F. poae*, and *F. equiseti*. Martin *et al.* (1991) also have isolated similar *Fusarium* spp. from wheat and barley (*Hordeum vulgare* L.) in Atlantic Canada.

The objective of this present study was to determine which *Fusarium* spp. infected wheat seeds at a site in Quebec and to see the effect of the plant growth regulator Cerone on *Fusarium* infection of four cultivars of spring wheat.

2.3. MATERIALS AND METHODS

Seed samples were obtained from a field trial conducted to test the effect of Cerone (Union Carbide AG Products,

Research Triangle, NC, USA) on the yield components of four cultivars of spring wheat (Columbus, Katepwa, Max, and Messier) at Macdonald Campus of McGill University in 1990. The plots were arranged in a randomized complete block design, with four blocks. Each plot was 3.8 m long, with 11 rows spaced 10 cm apart. Cerone was applied at a rate of 480 g/ha at Zadoks growth stage (ZGS) 39-45 (Zadoks *et al.*, 1974), just prior to boot swelling. The seeds were either hand or machine harvested.

A 50-seed sample was taken from each replication of the trial and was considered as one replicate. The experimental design was a 2x4 factorial experiment arranged in completely randomized design with four replicates. There were four cultivars and two growth regulator treatments (with and without Cerone).

The seeds were surface disinfested by soaking them in a 0.6% sodium hypochlorite solution (Javex) for about six minutes and then rinsing immediately by soaking them in three rinses of sterile water for 15 minutes each. The seeds were then blotted on sterile paper towels to drain the excess water.

Ten seeds were placed on a petri dish containing 'half-strength PDA + PCNB' medium. Five plates were used for each

replicate, and there were four replicates per treatment. This isolation medium was developed for the rapid sporulation of *Fusarium* spp., and contains potato dextrose broth (PDB)(Difco Laboratories, Detroit, Michigan, USA), 12.0 g (1/2 strength of PDA); agar, 20.0 g; PCNB (pentachloronitrobenzene), 1.5 g; chloramphenicol, 0.5 g; per litre of distilled water. The petri dishes were incubated at room temperature (22-24°C) for five days. *Fusarium* species were identified according to Nelson *et al.* (1983), Booth (1971), and Nirenberg (1981). The petri dishes were kept in a cold room (10°C) to inhibit the growth of *Fusarium* species until colonies could be identified. The number of seeds infected by each *Fusarium* spp. were counted, and expressed as a percentage of the total number of seeds.

The data obtained were analyzed according to the Statistical Analysis System (SAS Institute, 1985). An F-test was used to determine the significance of the variation caused by treatments and their interaction (Steel and Torrie, 1980). A protected least significant difference (LSD) test at the 0.05 level of significance was carried out to compare the differences among treatments. Orthogonal contrasts were used to test the treatment differences within the treatment interaction.

2.4. RESULTS

Using 'half-strength PDA + PCNB' medium, *Fusarium* species grown on culture formed mostly light to dark red colours on the underside of the colony and yellow to a lesser extent. The average percent seed infection by *Fusarium* spp. ranged from 1% to 11% in machine harvested seeds (Table 2.1), and from 0% to 4% in hand harvested seeds (Table 2.2). The most common species infecting the samples from machine harvested seeds (Table 2.1) were *Fusarium graminearum* (Schwabe), *F. poae* (Peck) Wollenw., *F. sporotrichioides* (Sherb), and *F. equiseti* (Corda) Sacc. The less common species were recorded under *Fusarium* spp. (Table 2.1). Different results were observed in samples from hand harvested seeds, in which the two dominant species were *F. sporotrichioides* and *F. poae,* while *F. graminearum* and *F. equiseti* were not detected (Table 2.2).

Significant differences in seed infections were observed among cultivar treatments, but not in Cerone treatments in the machine harvested seeds (Table 2.3). There was also a significant interaction between cultivar and Cerone treatment. In hand harvested seeds, significant differences were only detected among cultivar treatments, and no interactions were detected between cultivar and Cerone treatment. Therefore, the seed infection data was averaged over Cerone treatment, and presented in Figure 2.1.

Orthogonal contrasts show that higher seed infection from machine harvested seeds was observed in treatments with cultivar Columbus and Max, compared to Messier and Katepwa (Table 2.4). In hand harvested seeds, significant differences in seed infection were detected only in treatments with cultivar Columbus, and Cerone had no effect (Figure 2.1). In machine harvested seeds, a significant difference in seed infection between Cerone treatments and control treatment (without Cerone) was only observed in cultivar Columbus, in which 11% of seeds were infected with *Fusarium* spp. in the Cerone treatment, and only 3% in the control treatment (Figure 2.2).

Table 2.1. Average percent infection of machine harvested seeds of four cultivars of spring wheat with and without Cerone

Species	Cultivars							
	Max		Columbus		Messier		Katepwa	
	-c	+c	-c	+c	-c	+c	-c	+c
F. graminearum	1	0.5	0	3	0.5	0.5	0.5	0
F. sporotrichioides	3.5	1	0	4	1	0.5	0	0.5
F. poae	0.5	0.5	1	0.5	0	0	1	0
F. equiseti	0	0	0	0.5	0.5	0	0	0
F. spp.	0.5	2.5	2	3	0	2	0	0.5
Total	5.5	4.5	3	11	2	3	1.5	1

-c = Treatments without Cerone (0 g/ha).

+c = Treatments with Cerone (480 g/ha).

Table 2.2. Average percent infection of hand harvested seeds of four cultivars of spring wheat with and without Cerone

Species	Cultivars							
	Max		Columbus		Messier		Katepwa	
	-c	+c	-c	+c	-c	+c	-c	+c
F. sporotrichioides	0	0	0.5	0.5	0.5	0.5	0	0
F. poae	0	0.5	1	3	0	1	0.5	0.5
F. spp.	0	0.5	0	0	0	0	0.5	0
Total	0	1	1.5	3.5	0.5	1.5	1	0.5

-c = Treatments without Cerone (0 g/ha).

+c = Treatments with Cerone (480 g/ha).

Table 2.3. Source of variation and their significance from analysis of variance on seed infection by *Fusarium* species from machine and hand harvested seeds

Source	df	Machine harvested		Hand harvested	
		Mean Square	F	Mean square	F
Replicate	3	9.458	0.84 NS	1.125	0.66 NS
Cultivar	3	62.458	5.54 **	6.458	3.81 *
Cerone	1	15.125	1.34 NS	6.125	3.61 NS
Cv.x Cerone	3	39.125	3.47 *	2.125	1.25 NS
Error	21	11.268		1.696	

* = significant at P = 0.05

** = significant at P = 0.01

NS = not significant at P = 0.05.

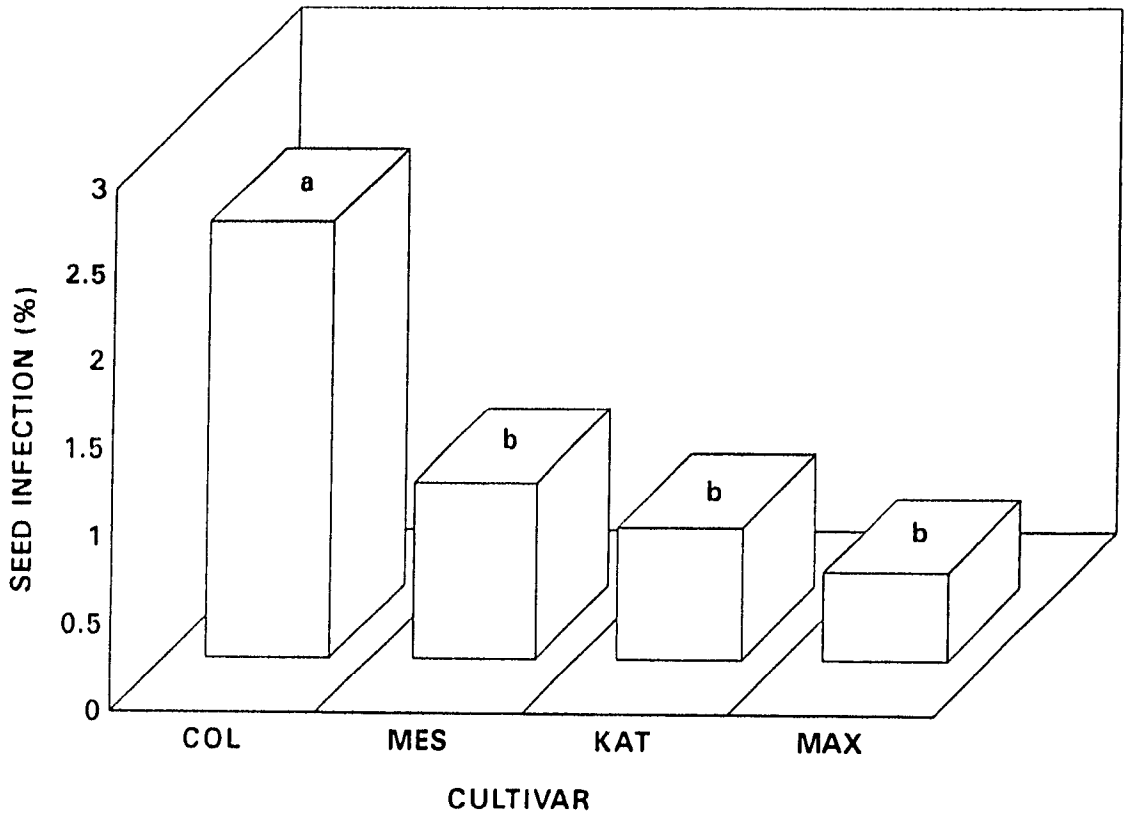

Figure 2.1. Seed infection by *Fusarium* species in four cultivars of spring wheat from hand harvested samples. Each bar represents the mean percent of seed infection. Bars with the same letter are not significantly different according to LSD analysis (P=0.05).

Table 2.4. Orthogonal contrast of interactions between Cerone and cultivar treatments on the percent infection of *Fusarium* species in machine harvested seeds

Contrast	df	Contrast SS	Mean Square	F Value
0 vs 480 in Col	1	128.00	128.00	11.36 **
0 vs 480 in Kat	1	0.50	0.50	0.04 NS
0 vs 480 in Max	1	2.00	2.00	0.18 NS
0 vs 480 in Mes	1	2.00	2.00	0.18 NS
Col & Max vs Kat & Mes	1	171.13	171.125	13.86 **

** = significant at P = 0.01

NS = not significant at P = 0.05.

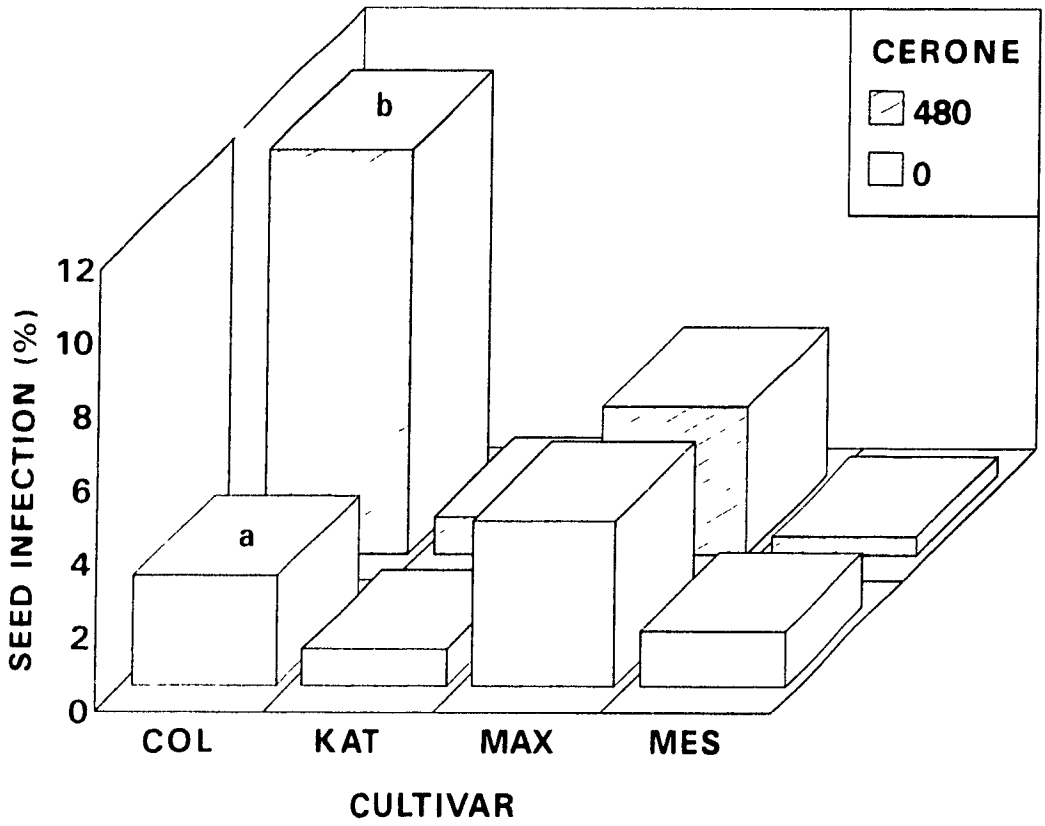

Figure 2.2. Effect of plant growth regulator Cerone on seed infection by *Fusarium* species in four cultivars of spring wheat, in samples of machine harvested seeds. Each bar represents the mean percent of seed infection. Bars with different letters are significantly different according to orthogonal contrast tests (P=0.01).

2.5. DISCUSSION

The average seed infection detected in samples from machine harvested seeds was higher than in samples from hand harvested seed. The difference might due to the small number of seeds in the hand harvested samples. The number of hand harvested seeds was around 200 seeds, a much smaller number compared to one kg or more seeds for machine harvested samples. Therefore, the discussion is mainly based on the results from machine harvested seeds.

The dominance of species of *Fusarium graminearum, F. sporotrichioides, F. poae,* and *F. equiseti* as the causal organisms of fusarium head blight (scab) in this survey was similar with those reported by Duthie *et al.* (1986), except that *F. avenaceum* was not detected. Martin *et al.* (1991) also found similar results, but *F. equiseti* was not detected in their work.

Among the predominant species, *F. sporotrichioides* contributed the highest level of average infection (10.5%), followed by *F. graminearum* (6%). *F. poae* and *F. equiseti* contributed 3.5% and 1% respectively. The lower infection by *F. graminearum* (6%) does not mean that the pathogen is not important as the causal agent of fusarium head blight of wheat. Duthie *et al.* (1986) stated that seed infections of 4%

are significant to the diseases of wheat. The 6% level of seed infection would result in infection of 3.42% to 4.20% of tiller bases.

The higher seed infections detected in cultivars Columbus and Max may due to the dwarf habit of these cultivars. Data from Western Bread Wheat Co-operative in 1978 and 1979 showed that the average height of Columbus was 82 cm (Anonymous, 1980), while data from Maritime-Quebec Coop Spring Tests in 4 years (1982-1985) showed that the average height of Max was 89.50 cm (Anonymous, 1987). The average height of cultivar Messier, on the other hand, is relatively taller (97 cm) than both cultivars (Anonymous, 1987). Our results agree with Mesterhazy (1991) who reported that one possible factor of resistance to fusarium head blight is tall plants.

The effect of the plant growth regulator Cerone on the seed infection was inconsistent. A significant difference was only observed in Columbus, in which 11% of the seeds were infected by *Fusarium* spp. in Cerone treatment, but only 3% in the treatment without Cerone. The inconsistent result of Cerone, which can release ethylene, on plant diseases has also been reported by other authors. Archer and Hislop (1975) have stated that ethylene can either increase or decrease plant susceptibility to fungal infection. The effect of Cerone on seed infection of wheat by *Fusarium* spp. is still unclear.

Therefore, further study is needed on the effect of Cerone and other plant growth regulators on fusarium head blight incidence of wheat.

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PREFACE TO CHAPTER 3

In Chapter 2 it was found that four predominant species infecting the seeds of spring wheat cultivars were *Fusarium graminearum*, *F. sporotrichioides*, *F. poae*, and *F. equiseti*. The plant growth regulator Cerone only increased seed infection on cultivar Columbus. However, the effect of Cerone on seed infection of wheat by *Fusarium* species was still unclear. The following chapter, Chapter 3, was designed to determine the effect of the growth regulators Cycocel and Cerone, and nitrogen fertilizer on fusarium head blight under greenhouse conditions. In these experiments, *F. graminearum*, a principal causal agent of fusarium head blight, was applied to cultivar Max, a susceptible cultivar widely grown in Quebec. Each table and figure is presented on the page following the first referral to it.

III. THE INFLUENCE OF PLANT GROWTH REGULATORS AND NITROGEN FERTILIZER ON FUSARIUM HEAD BLIGHT INCIDENCE OF WHEAT UNDER GREENHOUSE CONDITIONS

3.1. ABSTRACT

Hard red spring wheat cultivar Max growing under greenhouse conditions was treated with the plant growth regulators Cerone, Cycocel, or without at a rate equivalent to the recommended rates in the field and at appropriate Zadoks growth stages in winter 1990, and treated either with or without Cerone and nitrogen fertilizer in winter 1991. These experiments were conducted to investigate the effect of growth regulators and nitrogen application on the disease progress of *Fusarium graminearum*. At anthesis, heads were inoculated with a suspension of macroconidia (9000/ml), which had been shown previously to cause around 50% disease, and covered with plastic bags for 48 hours. Disease ratings, based on the number of infected spikelets per head, were assessed over the next three weeks. Area under the disease progress curve (AUDPC) was calculated and statistically analyzed. In two trials, there was no significant difference in disease progress between the treatments with and without growth regulators. No significant differences were also observed in disease progress among treatments with and without Cerone, nitrogen, or their interactions in two trials.

3.2. INTRODUCTION

Fusarium head blight, one of the major diseases of wheat (*Triticum aestivum* L.) in Canada is caused mainly by *Fusarium graminearum* Schwabe, the conidial state of *Gibberella zeae* (Schw.) Petch (Sutton, 1982). It can constrain yield as much as 50 percent (Jones and Clifford, 1983). Higher losses can occur when the fungus produces mycotoxins during the colonization of the seed. Contaminated seeds are unfit for human and animal consumption (Sutton, 1982). Moreover, Tuite *et al.* (1990) reported that the ability of infected seeds to germinate is decreased.

Nitrogen fertilization has been associated with the development of diseases incited by *Fusarium* species (Huber and Watson, 1974). Maric (1981) has stated that nitrogen application increased fusarium head blight incidence of wheat. Martin *et al.* (1991) also found similar increases in the incidence of seed infection of wheat and barley by *Fusarium* spp. in plots with supplementary nitrogen. However, Teich and Hamilton (1985) found no significant difference in head blight incidence between normal and extra nitrogen fertilizer levels.

Plant growth regulators, such as CCC [(2-chloroethyl) trimethylammonium chloride] and ethephon [(2-chloroethyl) phosphonic acid], have been used to reduce lodging, a negative

effect of heavy nitrogen fertilization (Dahnous *et al.*, 1982). The use of plant growth regulators, however, has been commonly associated with increased disease (Griffiths, 1981). Bockmann (1968) reported that CCC increased fusarium head blight incidence of wheat, while Martin *et al.* (1991) reported an increase in seed infection by *Fusarium* spp. after ethephon application. Michniewicz and Czerwinska (1991), however, in their in vitro studies reported that ethephon inhibited the growth of *Fusarium culmorum*, a causal agent of fusarium head blight.

Most studies on the effect of plant growth regulators on the incidence of fusarium head blight of wheat were conducted in the field with natural infections. The objective of the current study was to determine the effect of nitrogen fertilizer and plant growth regulators on fusarium head blight incidence of spring wheat under greenhouse conditions, with the inoculum applied directly to the heads.

3.3. MATERIALS AND METHODS

3.3.1. Inoculum density test

Preparation of plants. The seeds of cultivar Max were planted in plastic pots 12 cm diameter previously filled with 50

percent peat medium (Pro-Mix C, Premier Peat Co.) and 50 percent pasteurized greenhouse soil. Each pot was planted with three seeds, and placed on a temperature-controlled growth bench at 22° C. After two weeks, one uniform plant was left in each pot, and the pots were then transferred into the greenhouse.

Preparation of conidia suspension. A 6-mm^2 agar slice from the middle of a 5-day-old PDA culture of an isolate of *Fusarium graminearum* (isolate 7) was placed in a 1000 ml flask containing 500 ml of carboxymethylcellulose (CMC) medium (Cappellini and Peterson, 1965). The flask was then placed on the shaker for about 7 days. The conidia produced were harvested by filtering the suspension through two layers of sterile muslin cloth. The conidial density was determined by using a haemocytometer.

Inoculation of plants. A calibration experiment was first conducted to determine the volume of spore suspension that would be deposited on each head. In this experiment, heads were uniformly sprayed for five sec with a known concentration of conidia and the heads were placed in a flask with 99 ml of sterile water. The flasks were agitated on a rotary shaker for three minutes, and the suspension was dilution plated on Fusarium selective medium. From this test, it was established that approximately 70 μl of suspension was deposited on each

head. At anthesis, the main stem head of the plant was inoculated with inoculum density of 0, 20, 66, 200, 666, 2000, 6666, or 20000 conidia per head, which was equivalent to 0, 278, 926, 2778, 9258, 27778, 92583, or 277778 conidia/ml, using an artist's air brush (Badger-350). The heads were then covered with plastic bags and incubated at room temperature (22-24°C) for 48 hours. The bags were then removed, and the plants were placed in a greenhouse (16-25°C) with 16 hours of light, supplemented by sodium vapour lamps. Disease incidence was determined by counting the number of infected spikelets per head each day.

Experimental design and data analysis. The experimental design was a randomized complete block design with 12 replicates. A polynomial regression was used to describe the influence of inoculum density on disease incidence according to the Statistical Analysis System (SAS Institute, 1985).

3.3.2. Plant growth regulators

The seeds were planted in the same manner as in the previous experiment. At approximately Zadoks growth stage (ZGS) 31, when the first node was detectable (Zadoks *et al.*, 1974), 8 plants were sprayed with Cycocel (Cyanamid Canada Inc., Markham, Ontario) at the rate of 1.20 kg/ha in 345 L using an automatic spray chamber (Incom Int., Research

Instrument Mfg. Co. Ltd., Guelph, Ontario). The plants were transferred to the greenhouse and arranged on the bench. At approximately ZGS 39-45 when the fifth node was detectable until the boots were swollen, 8 more plants were sprayed with Cerone at the rate of 480 g/ha in 345 L. Eight untreated plants acted as controls. The plants received a 20-20-20 NPK commercial fertilizer (Peters, W. R. Grace Co.) every other week and were watered daily.

At anthesis, the plants were inoculated by spraying the main stem head using an artist's air brush containing a conidial suspension with a concentration of 9000 conidia/ml and one drop of Tween 20. The heads were immediately covered with plastic bags with wet paper towels in them to maintain high humidity. The plants were then kept at room temperature (22-24° C) for 48 hours. The bags were then removed, and the plants were placed in a greenhouse under the conditions described in the previous section 'Inoculation of plants'. During the three weeks after inoculation, the disease incidence was assessed by counting the percentage of infected spikelets per head.

The experiment was arranged in a randomized complete block design with 8 replicates. The experiment was conducted twice. Disease progress curves of the treatments were compared

by calculating the area under the disease progress curve (AUDPC). The AUDPC was estimated as:

$$AUDPC = \sum_{i}^{n-1} \frac{(Y_i + Y_{i+1})}{2} (t_{i+1} - t_i)$$

in which n is the number of assessment times, y is disease incidence, and t is time of assessment (Campbell and Madden, 1990).

The AUDPC was analyzed according to the Statistical Analysis System (SAS Institute, 1985). An F-test was used to determine the significance of the variation caused by treatments (Steel and Torrie, 1980). A protected least significant difference (LSD) test at the 0.05 level of significance was carried out to compare the differences among treatments.

3.3.3. Nitrogen fertilizer and Cerone

The seeds of cultivar Max were planted in plastic pots previously filled with soil as previously described. Each pot was planted with three seeds. After two weeks, one uniform plant was left in each pot. Sixteen plants were fertilized with ammonium nitrate in the form of 34-0-0 at the rate of 140 kg N/ha or approximately 0.034 g N/plant. Another 16 plants

were not given ammonium nitrate. At 39-45 ZGS, 16 plants (8 unfertilized and 8 fertilized plants) were sprayed with Cerone at the rate of 480 g/ha in 345 L using an automatic spray chamber. The other 16 plants were untreated with Cerone.

The plants were inoculated in the same manner as in the plant growth regulator experiment previously described. The plants were given no additional fertilizer in the course of the experiment. The experiment was conducted twice. The data obtained were analyzed in the same way as the previous experiment.

3.4. RESULTS

3.4.1. Inoculum density test

The incidence of fusarium head blight increased with increasing inoculum density (Figure 3.1). Fifty percent disease incidence level was obtained when the plants were inoculated with a conidial suspension of 666 conidia/head, which was equivalent to 9258 conidia/ml. The polynomial equation of the regression was of the form:

$$Y = 0.227 + 0.00024X - 0.0000000089X^2$$

where Y is arcsin transformation of disease incidence and X is the log (inoculum density + 1) transformation.

3.4.2. Plant growth regulators

All inoculated heads with inoculum density of 9000 conidia/ml showed the symptoms of fusarium head blight 5 days and 6 days after inoculation in experiments 1 and 2 respectively. The disease incidence ranged from 58% to 100% in experiment 1, with an average of 86%, and from 17% to 100% in experiment 2, with an average of 68%.

There were no significant differences observed in the disease progress of fusarium head blight among treatments with Cycocel, Cerone, or without growth regulators in experiment 1 (Figure 3.2 and Table 3.1). Similar results were also observed in experiment 2 (Figure 3.3 and Table 3.1).

3.4.3. Nitrogen fertilizer and Cerone

The symptoms of fusarium head blight were first observed 11 days and 6 days after inoculation in experiments 1 and 2 respectively. Disease incidence ranged from 27% to 86% in

experiment 1, with an average of 56%, and from 26% to 80% in experiment 2, with an average of 59%.

Nitrogen fertilizer in the form of ammonium nitrate and Cerone application did not influence the disease progress of fusarium head blight and no interactions were found among the main effects in either experiment (Table 3.2).

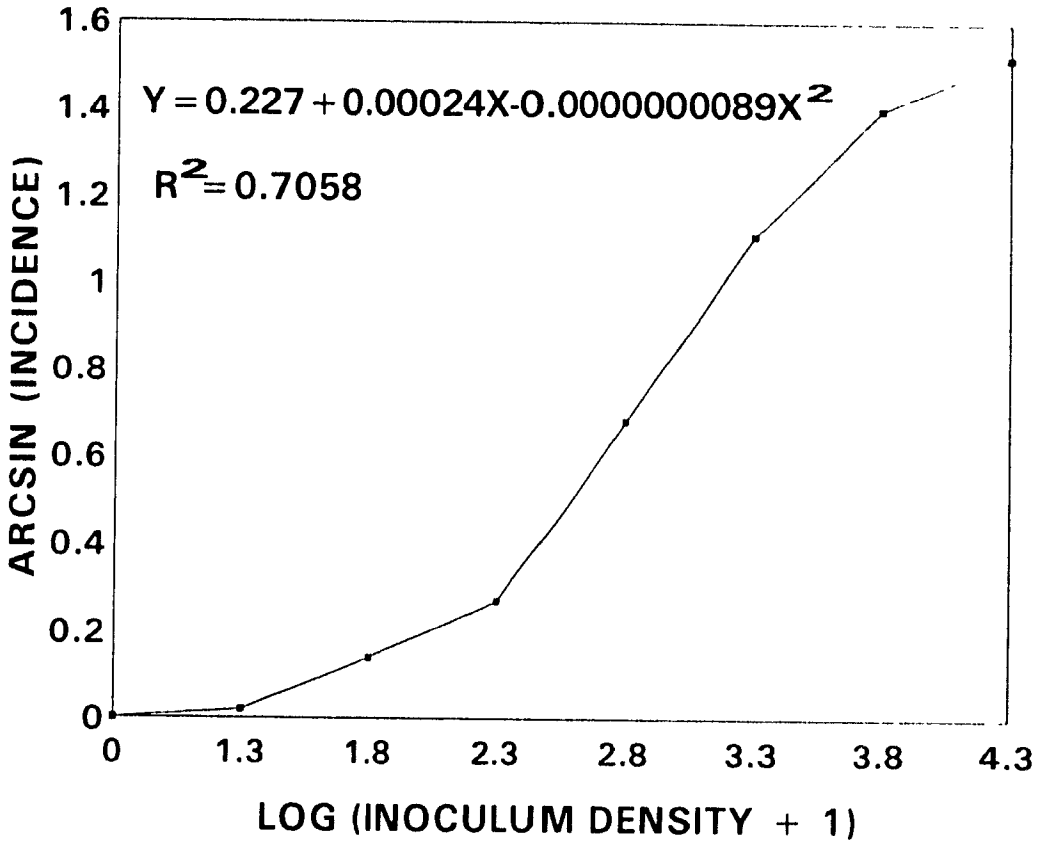

Figure 3.1. Effect of inoculum density of *Fusarium graminearum* on the incidence of fusarium head blight.

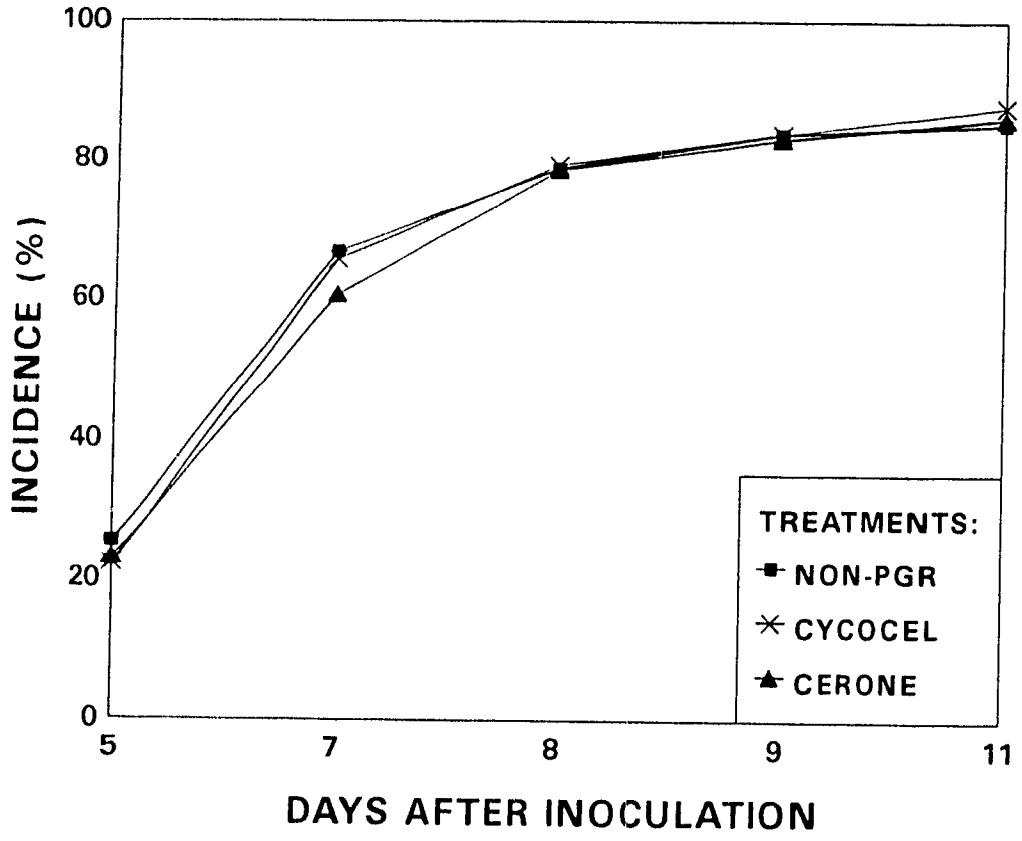

Figure 3.2. The effect of growth regulators on the disease progress of *Fusarium graminearum*, experiment 1.

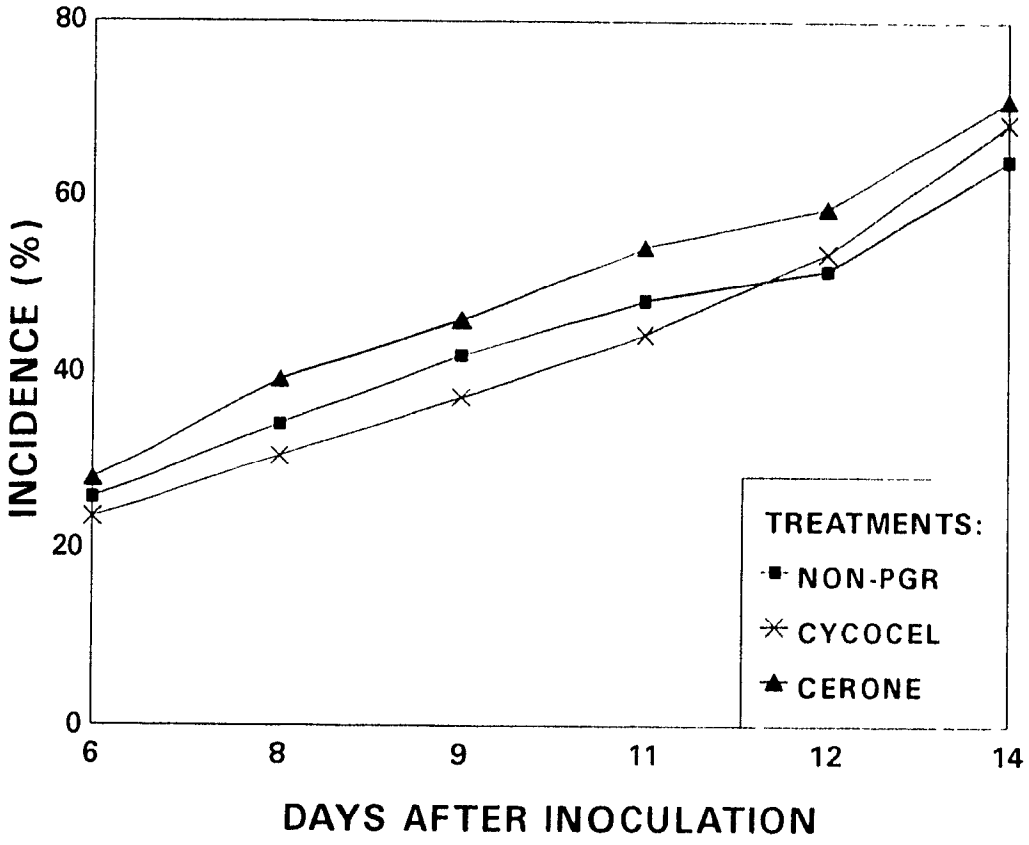

Figure 3.3. The effect of growth regulators on the disease progress of *Fusarium graminearum*, experiment 2

Table 3.1. Effect of growth regulators on the area under the disease progress curve of fusarium head blight

Source of variation	df	Mean square	F
Experiment 1			
Replicate	7	5129.159	0.82 NS
Treatment	2	651.407	0.10 NS
Error	14	6273.592	
Experiment 2			
Replicate	7	25855.783	1.87 NS
Treatment	2	10060.724	0.73 NS
Error	14	13791.923	

NS = not significant at P = 0.05

Table 3.2. Effect of a plant growth regulator and nitrogen fertilization on the area under the disease progress curve of fusarium head blight

Source of variation	df	Mean square	F
Experiment 1			
Replicate	7	27753.624	1.79 NS
PGR	1	7149.453	0.46 NS
N	1	23848.624	1.54 NS
PGR*N	1	900.298	0.06 NS
Error	16	15520.225	
Experiment 2			
Replicate	7	22847.418	0.97 NS
PGR	1	141.693	0.01 NS
N	1	4772.360	0.20 NS
PGR*N	1	427.415	0.02 NS
Error	16	23671.591	

NS = not significant at P = 0.05.

3.5. DISCUSSION

Hard red spring wheat cultivar Max is susceptible to fusarium head blight. A survey in Quebec in spring 1990 indicated that the highest percentage of infected spikelets by *Fusarium graminearum* was noted on cultivar Max (Devaux, 1991).

Neither of the plant growth regulators used significantly increased the disease progress of *F. graminearum*. This finding does not concur with those reported by Bockmann (1968) in which CCC application increased the susceptibility of wheat to infection by *Fusarium culmorum*. Further, Bockmann suggested that CCC application, which can shorten the plant, can favour development of the disease through three possible ways. In shortened plants, there is a shorter distance for the spore to travel from the soil to the ear, there is a more humid microclimatic condition near the soil surface, and the plant development is prolonged thereby extending the susceptible stage of infection. Martin *et al.* (1991) also proposed similar hypotheses explaining the increase in seed infections as a result of ethephon application. The non significant difference in disease progress after Cycocel and Cerone treatments in our work may have resulted from the inoculation method, in which inoculum was sprayed directly to the head at the most susceptible stage (anthesis). The condition in the greenhouse,

with 48 hours of uninterrupted wetness after inoculation also favoured the rapid development of severe disease.

The use of ammonium nitrate in this work was based on Teich (1987), who reported that fusarium head blight incidence was higher when wheat was fertilized with ammonium nitrate rather than urea. However, our results showed that nitrogen application did not significantly increase fusarium head blight incidence. These results agree with Teich and Hamilton (1985), in which no significant difference in head blight incidence was observed with different levels of nitrogen fertilizer.

There were no significant differences observed in the disease progress of *F. graminearum* when inoculum was applied to the head in the greenhouse condition. However, other authors found different results in the field from natural infections. Therefore, it is worthwhile to study the effect of growth regulators on fusarium head blight incidence in the field with different methods of artificial inoculations.

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PREFACE TO CHAPTER 4

The results of Chapter 3 showed that none of the plant growth regulators influenced the disease progress of *Fusarium graminearum* in trials conducted under controlled greenhouse conditions. Nitrogen fertilization also did not affect the progress of the disease. The macroconidia of *F. graminearum* were applied directly to the heads. The following chapter, Chapter 4, was designed to investigate the effect of plant growth regulators and nitrogen fertilization on the incidence of fusarium head blight with different methods of artificial inoculation and different types of inoculum in the field. The plots were inoculated with macroconidia of *F. graminearum* or with *Fusarium*-colonized corn (ascospores of *Gibberella zeae*). The colonized corn was used because it more closely approximates the natural epidemiology of this disease in Eastern Canada. Each table and figure is presented on the page following the first referral to it.

IV. THE EFFECT OF GROWTH REGULATORS AND NITROGEN FERTILIZER ON FUSARIUM HEAD BLIGHT OF WHEAT IN THE FIELD

4.1. ABSTRACT

The effect of the growth regulators Cerone and Cycocel on head blight of spring wheat cultivar Max was tested in irrigated field trials inoculated with *Fusarium graminearum* in 1991 in Quebec, and in 1992 in Ontario. The effect of nitrogen fertilization was also tested in the 1991 trial. In 1991, a dry year, there were no symptoms of head blight, but the incidence of seed infection ranged from 2 to 20 % in treatments where heads were inoculated with macroconidia of *F. graminearum*. Cycocel treatments had the highest incidence of infection, but were not significantly different from the non-treated control ($P = 0.06$). No significant differences were observed in the incidence of seed infection between treatments with or without nitrogen. In 1992, plots were inoculated with macroconidia sprayed on the heads or with *Fusarium*-colonized corn applied in the rows. Three weeks after application, mature perithecia were found on the colonized corn. The incidence of spikelet infection ranged from 2-4 % in the non-inoculated treatments to 7-22 % in the inoculated treatments, and the average incidence of seed infection ranged from 12-31 % in the non-inoculated treatments to 74-85 % in the inoculated treatments. Cycocel consistently increased disease

only in treatments with colonized corn applied to the rows. Cycocel also increased the incidence of seed infection in the non-inoculated treatments. Growth regulators had no effect on disease when heads were inoculated directly with macroconidia.

4.2. INTRODUCTION

Production of high quality wheat is the major concern in most wheat-producing countries. Quality of wheat can be restricted by the presence of fusarium head blight, which can cause inferior quality and low grade of grain, and can produce mycotoxins hazardous to humans and livestock (Sutton, 1982; Sydenham *et al.*, 1989; Teich, 1989).

Fusarium graminearum Schwabe, the conidial state of *Gibberella zeae* (Schw.) Petch, is the principal species associated with fusarium head blight of wheat in Canada (Sutton, 1982). In Quebec, the pathogen has infected all of the cultivars surveyed, with the highest infection (5.2%) on cultivar Max (Devaux, 1991).

Cultural practices have been associated with differences in incidence of fusarium head blight of wheat (Teich and Hamilton, 1985). Maric (1981) and Martin *et al.* (1991) reported that nitrogen fertilization can increase the

incidence of fusarium head blight. Growth regulators, used to reduce lodging, a negative effect of high nitrogen fertilization, have also been shown to increase plant diseases (Bockmann, 1968; Griffiths, 1981; Martin *et al.*, 1991).

The most susceptible stage of the plant for infection by *F. graminearum* is at the time of anthesis (Cook, 1981), which lasts only for about 3-5 days (Cook and Veseth, 1991). Macroconidia and ascospores produced on plant debris are the main source of inoculum (Sutton, 1982; Wiese, 1977), but ascospores are more common than macroconidia in humid climates (Cook, 1981; Reis, 1988). Ascospores are discharged from perithecia and mainly dispersed by wind (Sutton, 1982).

Most studies concerning the effect of growth regulators on fusarium head blight incidence were conducted in the field with natural infection. In those studies, it was not known whether the form of inoculum was macroconidia or ascospores. The objective of the present work was to investigate the effect of plant growth regulators and nitrogen fertilization on the incidence of fusarium head blight with different methods of artificial inoculation and different types of inoculum in the field.

4.3. MATERIALS AND METHODS

4.3.1. 1991 experiment

Field studies were conducted using hard red spring wheat cultivar Max in a fine sandy loam soil at Macdonald Campus of McGill University in Ste-Anne-de-Bellevue, Quebec. The experiment was a 3 x 3 x 2 factorial arranged in a randomized split-split plot design with four replicates. The main plots were inoculation treatments, the sub-plots were growth regulator treatments, and the sub-sub plots were nitrogen treatments. Each plot was 3.8 m long, with 11 rows spaced 10 cm apart. Plots were seeded using a tractor seeder at a rate of 450 seeds/m^2.

The plots were treated with Cycocel at a rate of 1.2 kg/ha at Zadoks growth stage (ZGS) 31 (Zadoks *et al.*, 1974) when the first node was detectable, Cerone at the rate of 480 g/ha at ZGS 39-45 when the fifth node was detectable until the boots were swollen, or no plant growth regulator. The plots were either fertilized with ammonium nitrate at the rate of 140 kg N/ha a week after seeding or were not fertilized. The plots were inoculated with macroconidia of *F. graminearum* isolate 7 with a conidial density of 6 x 10^5 conidia/ml at anthesis, a week after anthesis, or without inoculation. The plots were irrigated with a lawn sprinkler for 15 minutes

every two hours during the day, during the two weeks after inoculation.

Disease assessment was carried out by plating out 100 seeds from each plot on petri dishes containing 'half-strength PDA + PCNB', a selective medium. This isolation medium was developed for the rapid sporulation of *Fusarium* spp., and contained potato dextrose broth (PDB)(Difco Laboratories, Detroit, Michigan, USA), 12.0 g (1/2 strength); agar, 20.0 g; pentachloronitrobenzene (PCNB), 1.5 g; chloramphenicol, 0.5 g; per litre of distilled water. Seeds were surface disinfested as described in Section 2.3. The seeds on the medium were incubated at room temperature (22-24°C) for five days. Identification of *F. graminearum* was based on Nelson *et al.* (1983). The petri dishes were kept in a cold room (10°C) to inhibit the growth of the pathogen until colonies could be identified. The numbers of seeds infected by the pathogen were counted, and expressed as a percentage of the total number of seeds.

The data obtained were analyzed according to the Statistical Analysis System (SAS Institute, 1985). An F-test was used to determine the significance of the variation caused by treatments and their interactions (Steel and Torrie, 1980). A least significant difference (LSD) test at the 0.05 level of

significance was carried out to compare the differences among treatments.

4.3.2. 1992 experiment

A field experiment was conducted to determine the effect of the growth regulators Cerone and Cycocel with different methods of artificial inoculation. This experiment was conducted in a sandy loam soil at Central Experimental Farm in Ottawa, Ontario. The field trials were conducted there because of the superior irrigation and mist system, part of an existing Fusarium nursery plot for evaluating cultivar resistance. The experiment was a 3 x 3 factorial arranged in a split plot design with four replicates. The main plots were inoculation treatments, and the sub-plots were growth regulator treatments. Each plot was 3 m long, with 8 rows spaced 23 cm apart. The plots were not fertilized with nitrogen since the nitrogen content of the soil was high (100 kg N/ha). The plots were treated with herbicide Bromoxynil, common name: Buctril-M, (Rhone Poulenc Canada Inc., Missisauga, Ontario) to control weeds.

The plots were treated with Cycocel, Cerone, or without plant growth regulator in the same manner as the 1991 experiment. The plots were inoculated by spraying the heads using a backpack sprayer containing macroconidia with a

conidial density of 6 x 10^5 conidia/ml at anthesis on July 20, 1992, by spreading *Fusarium*-colonized corn (ascospore inoculation) in the rows at ZGS 31 on June 10, 1992; or were not inoculated. *F. graminearum* isolate DAOM 178148 (Biosystematics Research Centre, Agriculture Canada, Ottawa) was used in this field trial, since it produced more abundant perithecia than isolate 7, which was used the previous year. The *Fusarium*-colonized corn was produced by inoculating autoclaved corn kernels in jars with 10 ml of macroconidial suspension of *F. graminearum*. The macroconidial suspension was produced in a 100 ml flask containing 500 ml of carboxymethylcellulose (CMC) medium (Cappellini and Peterson, 1965). The jars were incubated in an incubator (20°C) under long-wave UV and fluorescent lamps for about two months. The colonized corn kernels were broken down before spreading them in the rows. Each plot received two jars of corn (500 g). After three weeks in the field, the corn kernels were covered with purple-black perithecia of *Gibberella zeae*, which contained mature asci and ascospores.

The plots were irrigated using an automatic mist system from 6 am to 8 pm everyday for 30 second every five minutes (2.7 gal/hr emitter) after macroconidia inoculation until the disease was assessed. The mist system was equipped with a sensor that turned off the system under wet conditions such as rain.

The disease was assessed on August 5, 1992 by counting the number of infected spikelets per head. Fifty heads were chosen randomly from the middle of each plot. Disease assessment was also conducted by plating out 100 seeds from each plot on '1/2 strength PDA + PCNB medium' as described previously in the 1991 experiment.

The data obtained were analyzed in the same manner as previously described in the section '1991 experiment'. Orthogonal contrasts were used to test the treatment differences within the treatment interaction.

4.4. RESULTS

4.4.1. 1991 experiment

Summer 1991 was a dry year. During the two-week period after inoculation (6 July-20 July), there was only 11 mm of rain, with high temperatures (above 30°C during the later part of that period). Symptoms of fusarium head blight were not observed in the field, but the incidence of seed infection ranged from 2 to 20% in treatments where heads were inoculated with macroconidia.

No significant differences were observed between treatments with or without nitrogen (Table 4.1). Cycocel treatments had the highest incidence of seed infection, but were not significantly different from the non-treated control (P=0.06)(Table 4.1). The only significant difference was observed in inoculation treatments, where non-inoculated treatments were significantly lower than inoculated treatments, but no significant differences were observed between the inoculation experiments regardless the time of inoculation (Figure 4.1).

4.4.2. 1992 experiment

This year was much wetter than 1991. During July, rainfall was approximately 150 % of the normal average. In the *Fusarium*-colonized corn treatments, mature perithecia were found three weeks after application. Incidence of spikelet infection (reading from the symptoms) ranged from 2-4 % in the non-inoculated treatments to 7-22 % in the inoculated ones. The average incidence of seed infection ranged from 12-31 % in the non-inoculated treatments to 74-85 % in the inoculated treatments.

Significant differences in the incidence of spikelet infection were observed among inoculation and growth regulator

treatments (Table 4.2). There was also a significant interaction between inoculation and growth regulator treatments. Orthogonal contrasts showed a higher incidence of spikelet infection in treatments with growth regulators, compared to non-growth regulator treatments when the plots were inoculated with *Fusarium*-colonized corn (Figure 4.2).

Significant differences were also observed in the incidence of seed infection among inoculation and growth regulator treatments (Table 4.3), but no interactions were detected between inoculation and growth regulator treatments. Therefore, the incidence of seed infection data was averaged over inoculation and growth regulator treatments, and presented in Table 4.4 and Figure 4.3. Cycocel increased seed infection compared to the non-treated control, but only in treatments inoculated with infected corn. Cycocel also increased seed infection in the non-inoculated treatment.

There were significant differences in plant height between growth regulator treatments (Table 4.5), but no interactions were detected between growth regulator and inoculation treatments. Therefore, the plant height data were averaged over growth regulator treatments, and there was a trend that the higher the plants the lower the incidence of seed infection (Table 4.6).

Table 4.1. Effect of growth regulators, nitrogen fertilizer, and time of inoculation on the incidence of seed infection by *F. graminearum* in the 1991 field experiment

Source of variation	df	Mean square	F value
Replicate	3	117.789	9.73 **
Inoculation	2	555.280	45.86 **
R*Inoculation	6	40.151	3.32 **
PGR	2	35.889	2.96 NS
Ino*PGR	4	11.066	0.91 NS
Nitrogen	1	0.084	0.01 NS
Ino*N	2	35.167	2.90 NS
PGR*N	2	14.011	1.16 NS
Ino*PGR*N	4	10.945	0.90 NS
Error	42	12.108	

** = significant at P=0.01

NS = not significant at P=0.05

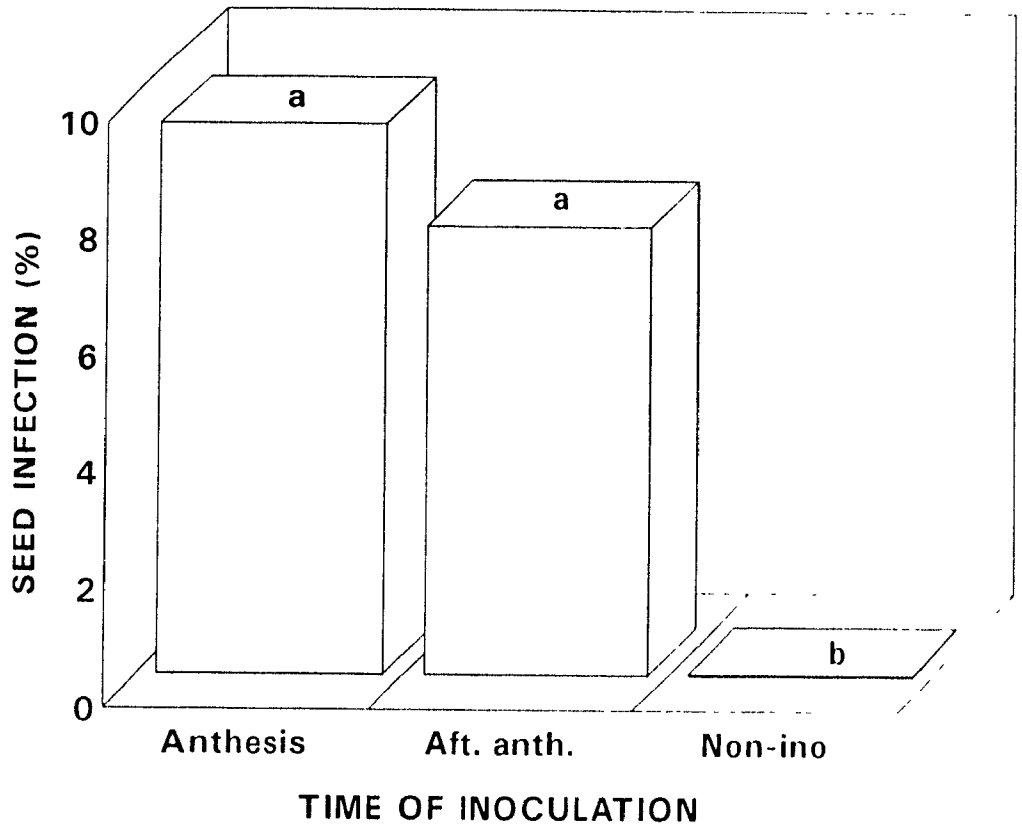

Figure 4.1. Effect of time of inoculation on the incidence of seed infection by *F. graminearum* in the 1991 field experiment. Bars with the same letters are not significantly different according to LSD tests (P=0.05). Anthesis = inoculated at anthesis. Aft. anth.= inoculated one week after anthesis. Non-ino = non-inoculated.

Table 4.2. Effect of growth regulators and method of inoculation on the incidence of spikelet infection by *F. graminearum* in the 1992 field experiment

Source of variation	df	Mean square	F value
Replicate	3	47.920	25.76 **
Inoculation	2	426.056	229.04 **
R*Ino	6	17.944	9.65 **
PGR	2	10.302	5.54 *
Ino*PGR	4	12.581	6.76 **
Error	18	1.860	

* = significant at P=0.05

** = significant at P=0.01

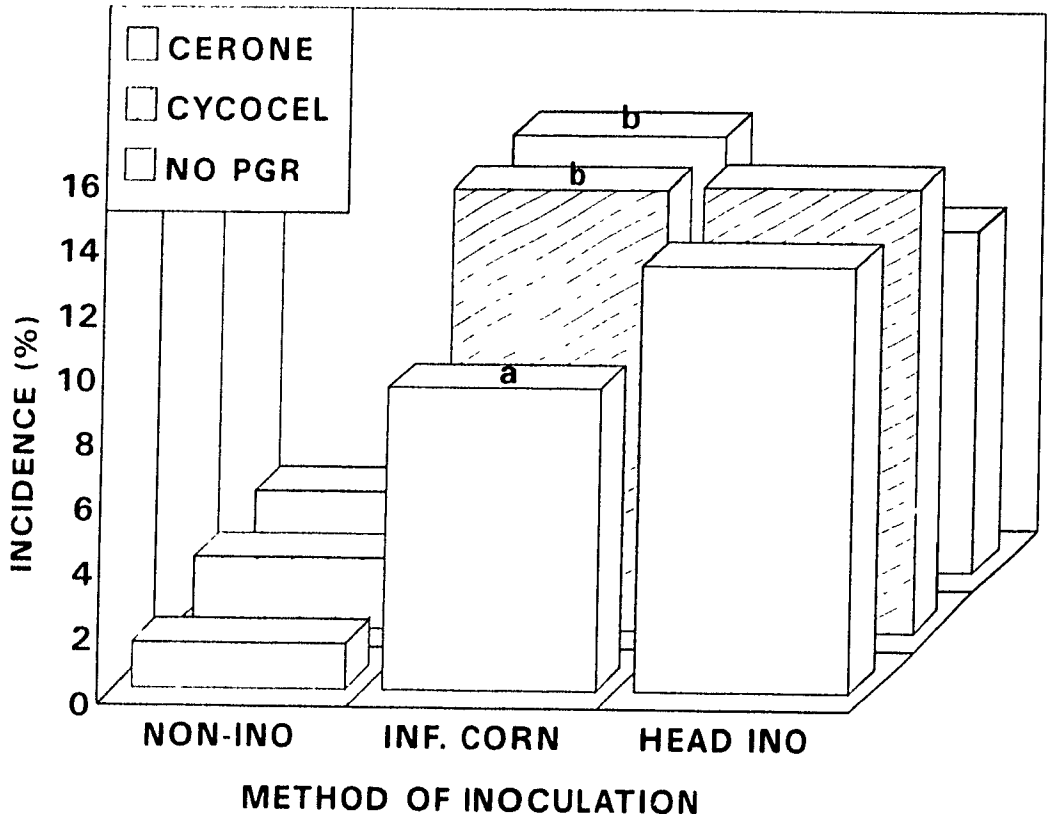

Figure 4.2. Effect of growth regulators and method of inoculation on the the incidence of spikelet infection by *F. graminearum* in the 1992 field experiment. Bars with the same letters are not significantly different according to orthogonal contrast tests (P=0.05). Non-ino = non-inoculated. Inf. corn = rows inoculated with *F. graminearum*-colonized corn, that later produced perithecia and ascospores. Head ino = heads inoculated with macroconidia of *F. graminearum*.

Table 4.3. Effect of growth regulators and method of inoculation on the incidence of seed infection by *F. graminearum* in the 1992 field experiment

Source of variation	df	Mean square	F value
Replicate	3	181.148	5.25 **
Inoculation	2	14155.750	410.42 **
R*Ino	6	76.454	2.22 NS
PGR	2	401.583	11.64 **
Ino*PGR	4	65.833	1.91 NS
Error	18	34.491	

* = significant at P=0.05

** = significant at P=0.01

NS = not significant at P=0.05

Table 4.4. The incidence of seed infection by *F. graminearum* averaged over growth regulators and method of inoculation in the 1992 field experiment

Growth regulator	Incidence (%)	Inoculation	Incidence (%)
Cycocel	65.417 a[1)]	Inf. corn	81.083 a[1)]
Cerone	58.083 b	Macroconidia	76.833 a
No PGR	54.000 b	Non-ino	19.583 b

1) = Figures in the same column followed by the same letters are not significantly different according to LSD tests (P=0.05).

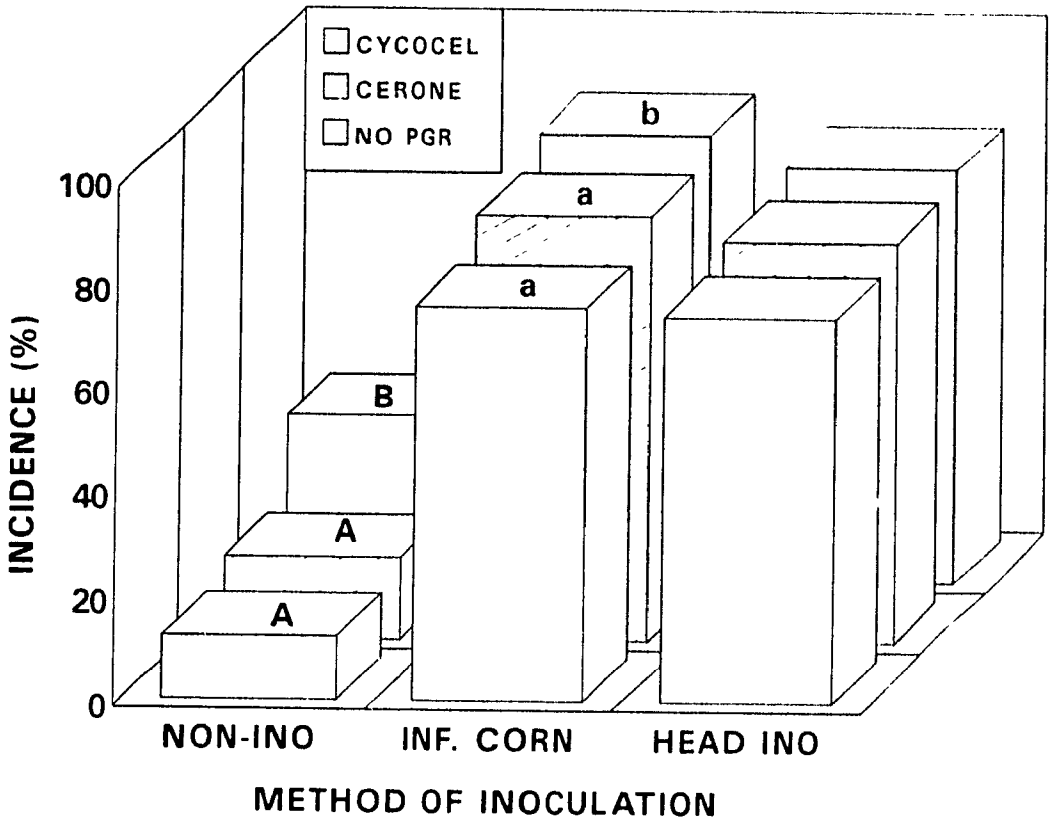

Figure 4.3. Effect of growth regulators and method of inoculation on the incidence of seed infection by *F. graminearum* in the 1992 field experiment. Bars with the same letters are not significantly different according to orthogonal contrast tests (P=0.05). Non-ino = non-inoculated. Inf. corn = rows inoculated with *F. graminearum*-colonized corn, that later produced perithecia and ascospores. Head ino = heads inoculated with macroconidia of *F. graminearum*.

Table 4.5. Effect of growth regulators and methods of inoculation on plant height in the 1992 field experiment

Source of variation	df	Mean square	F value
Replicate	3	32.259	4.92 *
Inoculation	2	18.325	2.80 NS
R*Ino	6	11.830	1.81 NS
PGR	2	1040.337	158.80 **
Ino*PGR	4	3.206	0.49 NS
Error	18	6.551	

* = significant at P=0.05

** = significant at P=0.01

NS = not significant at P=0.05

Table 4.6. Effect of growth regulators on plant height and the incidence of seed infection in the 1992 field experiment

Growth regulator	Plant height (cm)	Incidence (%)
No PGR	90.194 a[1)]	54.000 b[1)]
Cerone	77.947 b	58.083 b
Cycocel	71.922 c	65.417 a
LSD	2.195	5.037

1) = Figures in the same column followed by the same letters are not significantly different according to LSD tests (P=0.05)

4.5. DISCUSSION

Nitrogen fertilizer did not affect the incidence of fusarium head blight of spring wheat in the 1991 field trial. The results of this work agree with those reported by Teich and Hamilton (1985) who found that nitrogen level (normal or extra dose) did not influence head blight incidence. The form of nitrogen used in our work was ammonium nitrate. Teich (1987) reported a higher incidence of fusarium head blight when wheat was fertilized with ammonium nitrate rather than urea. Other forms of nitrogen may have different results.

None of the growth regulators used influenced the incidence of fusarium head blight in the 1991 field experiment. These results do not concur with those reported by Bockmann (1968), who found that CCC increased head blight incidence, and by Martin *et al.* (1991), who reported similar increases with ethephon both under condition of natural inoculation. The authors suggested that increases in fusarium head blight after plant growth regulator application may due to the reduction of plant height, which can cause changes in the microclimate of the canopy. The changes in microclimate may favour the development of the pathogen in plant debris. Growth regulators may also prolong the susceptible stages of the plants to the Fusarium infection. The non-significant effect of Cycocel and Cerone on fusarium head blight incidence

of wheat in our results may have seen due to the method of inoculation, in which macroconidia were directly applied to the heads at anthesis, the most susceptible stage of the plants (Cook, 1981; Sutton, 1982). This method of inoculation would override the effects of plant architecture and microclimate.

In 1992, the field plots were inoculated with macroconidia or with *Fusarium*-colonized corn, from which ascospores were ejected. Ascospores of *Gibberella zeae* may have a more important role in causing head blight of wheat than do macroconidia (Cook, 1981). Reis (1988) trapped spores above soil cultivated with wheat, and found that 98 % were ascospores and 2 % were macroconidia. Our observations showed that macroconidia and ascospores can cause a greater than 75 % incidence of fusarium head blight on wheat plants in the field. The method of inoculation using *Fusarium*-colonized corn more closely approximates the natural epidemiology of this disease in Eastern Canada (Sutton, 1982), and should be more extensively used in cultivar resistance trials. This method is easy, and does not require extensive technical facilities or exotic media to produce the inoculum.

The plant growth regulators Cerone and Cycocel significantly increased the incidence of spikelet infection when the *Fusarium*-colonized corn was applied in the rows, but

no effect was observed when the heads were sprayed with macroconidia. The plant growth regulators may increase the incidence of spikelet infection by *F. graminearum* as a result of the reduction in plant height (Mesterhazy, 1991), so that the distance required for spores to travel was shorter. This work has shown that growth regulators significantly decreased the plant height, and there was a trend that the shorter the plants the higher the disease incidence.

Cycocel but not Cerone significantly increased the incidence of seed infection and only in treatments inoculated with colonized corn. This finding concurs with those reported by Bockmann (1968), in which CCC (Cycocel) application increased the susceptibility of the plant to *Fusarium* infection. This increase in disease incidence may result from the effect of Cycocel on plant height and the prolongation of the susceptible stages of the plant.

The increase of fusarium head blight incidence by growth regulators in treatments with colonized corn (ascospores) suggests that managing plant debris as a source of inoculum will be very important in reducing fusarium head blight incidence of wheat in crops treated with plant growth regulators or when dwarf cultivars are used.

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V. GENERAL DISCUSSION AND CONCLUSIONS

Intensive cereal management (ICM) systems have been used successfully to increase the yield and to improve the quality of wheat (Frederick and Marshall, 1978). One of the important strategies in this management practice is a heavy nitrogen fertilization. Since high levels of nitrogen fertilization have been associated with increasing lodging intensity (Pumphrey and Rubenthaler, 1983), it is necessary to apply plant growth regulators as anti-lodging agents (Dahnous *et al.*, 1982). However, Martin *et al.* (1991) reported that supplementary nitrogen and a plant growth regulator can increase the incidence of seed infection by *Fusarium* species.

Fusarium head blight is one of the most destructive diseases of wheat in Canada (Sutton, 1982). In the first part of this report, it was found that four predominant species colonizing the heads of wheat were *Fusarium sporotrichioides*, *F. graminearum*, *F. poae*, and *F. equiseti*. It was also noted that the plant growth regulator Cerone increased the incidence of seed infection only on cultivar Columbus, a semidwarf cultivar. These findings agree with those reported by Mesterhazy (1991), in which taller plants were more susceptible to fusarium head blight than the shorter ones.

Studies on the effect of growth regulators and nitrogen fertilization on fusarium head blight incidence conducted in this report were made using cultivar Max, a susceptible cultivar widely grown in Quebec (Devaux, 1991), with *F. graminearum*, the major causal agent of fusarium head blight (Cunfer, 1987; Sutton, 1982).

In greenhouse trials, nitrogen application (ammonium nitrate) did not influence the disease progress of *F. graminearum*. Similar results have been reported by Teich and Hamilton (1985). No significant effect on the disease progress was also observed in either Cycocel or Cerone treatments. These results did not concur with those reported by Bockmann (1968) and Martin *et al.* (1991). The lack of significant differences in this experiment may due to the method of inoculation, in which macroconidia were sprayed directly onto the heads at anthesis, the most susceptible stage of the plant to *F. graminearum* infection. The conditions in the greenhouse with 48 hours of uninterrupted wetness after inoculation were very favourable for the development of high levels of disease.

In 1991, a dry year, no symptoms of head blight were observed in the field, but the incidence of seed infection ranged from 2 to 20% in the inoculated plots. The highest incidence was observed in Cycocel treatments, but this was not significantly different from the non-treated control (P=0.06).

Nitrogen fertilization also did not affect the incidence of seed infection. Martin *et al.* (1991) suggested that the plant growth regulator may increase disease incidence as a result of reduction in plant height. Reduction in plant height can increase the density of the canopy, which in turn can cause changes in microclimate that favour the development of the pathogen. Shorter plants also decrease the distance that spores must travel from the plant debris to the heads. Since the macroconidia were applied directly to the heads in our experiment, this method would override the effect of plant architecture and microclimate.

In 1992, both Cycocel and Cerone increased the spikelet infection, but only in the *Fusarium*-colonized corn treatments in which the inoculum was ascospores released from sexual structures formed on the colonized corn. The plant growth regulators may increase spikelet infection due to the reduction in plant height, so that the distance of ascospore travel from the colonized corn to the heads was shorter. Shorter heads may encounter a higher load of ascospores. This experiment showed that growth regulators significantly decreased the plant height and there was a trend that the shorter the plant the higher the disease incidence. Mesterhazy (1991) stated that shorter plants were more susceptible to fusarium head blight. Only Cycocel increased seed infection in the colonized corn treatments. This finding agrees with those

reported by Bockmann (1968), in which Cycocel application increased the susceptibility of the plant to *Fusarium* infection. The higher incidence of seed infection may result from the effect of Cycocel on the prolongation of the susceptible stages of the plant and on the reduction of plant height.

The increased incidence of fusarium head blight in plants treated with growth regulators in treatment with colonized corn suggests that managing plant debris as a source of inoculum will be very important in reducing fusarium head blight incidence of wheat. Breeding of resistant cultivars should stress the shortening of the susceptible stage of the plants (anthesis), while dwarf varieties should be evaluated in the light of their possible increased susceptibility to *F. graminearum*. Finally, the inoculation method using *Fusarium*-colonized corn should be used more extensively in cultivar resistance trials, since it more closely approximates the natural epidemiology of this disease. This method is easy, and does not require extensive technical facilities or exotic media to produce inoculum.

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