

**Effect of kaolin on the striped cucumber beetle (*Acalymma vittatum*)  
and cucumber growth and development.**

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## **Abstract**

### **Effect of kaolin on the striped cucumber beetle (*Acalymma vittatum*) and cucumber growth and development**

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M.Sc. Thesis, McGill University, 2007

Striped cucumber beetle (SCB) is the main pest of cucurbits in northeastern America. This project examined the efficacy of kaolin clay in controlling SCB (*Acalymma vittatum*; Coleoptera: Chrysomelidae) in cucumbers. Field experiments compared kaolin (Surround WP), insecticide (carbaryl; Sevin XLR) and untreated controls. In 2005, mean number of beetles was lowest in the kaolin treatment. Bacterial wilt (*Erwinia tracheiphila*) was greater in the controls and kaolin plants had significantly higher marketable yields than the two other treatments. In 2006, SCB numbers in kaolin were similar to the other treatments. Total marketable yield was significantly higher in the insecticide than the other treatments. Single and multiple applications of kaolin had negative short term effects on gas exchange and only negligible effects on greenhouse grown plants. In behavior experiments, kaolin reduced settling and feeding damage by SCB on treated plants. Kaolin shows potential as an alternative to insecticide especially to protect seedlings and young plants.

## Résumé

### **Effet du kaolin sur la chrysomèle rayée du concombre (*Acalymma vittatum*) et sur la croissance et le développement du concombre**

Geneviève Legault

Mémoire de maîtrise (M.Sc.), Université McGill, 2007

La chrysomèle rayée du concombre (CRC) est le principal insecte ravageur des cucurbitacées. L'efficacité du kaolin pour contrôler la CRC (*Acalymma vittatum*) a été testée dans une culture de concombres. Un essai en champ de deux ans a comparé le kaolin (Surround WP) à un insecticide (carbaryl; Sevin XLR) et un témoin non traité. En 2005, la moyenne saisonnière de CRC était inférieure dans le kaolin. Le flétrissement bactérien (*Erwinia tracheiphila*) était plus abondant dans le témoin et le kaolin avait des rendements vendables significativement supérieurs aux autres traitements. En 2006, la moyenne de CRC dans le kaolin était comparable aux autres traitements. Le rendement vendable était significativement supérieur dans les parcelles traitées à l'insecticide. Une ou plusieurs applications de kaolin ont eu des effets négatifs sur les échanges gazeux de la feuille à court terme mais des effets négligeables sur la croissance des plants en serre. Lors de l'étude du comportement, le kaolin a réduit la présence des CRC et les dommages sur le feuillage. Le kaolin montre un bon potentiel comme alternative aux insecticides pour protéger les jeunes plants de cucurbitacées.

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## Introduction

Striped cucumber beetle (SCB), *Acalymma vittatum* (Fabricius) is the most important pest of cucurbits in northeastern America. SCB completes its life cycle on plants of the family Cucurbitaceae; the larvae feed exclusively on cucurbit roots (Munroe and Smith 1980) and the adults feed on foliage, stems, flowers and fruit. In addition, the SCB adult transmits bacterial wilt, *Erwinia tracheiphila* (Smith), cucumber mosaic, cowpea mosaic and pumpkin mosaic viruses (Munroe and Smith 1980; Pitblado and Lucy 1994). Pitblado and Lucy (1994) reported yield losses of 15% from feeding damage of SCB in cucurbits fields in Canada. Furthermore, *E. tracheiphila* transmitted by SCB induced additional yield losses that can be up to 75% of the crop (Ellers-Kirk 1996).

The most common control method for the SCB is the use of insecticides. However, increased insect resistance to pesticides, as well as health concerns coupled with the growing interest in organic agriculture have raised interest in alternative control methods. To develop a successful IPM strategy for SCB, a number of biological and cultural alternatives have been examined. Recently, the use of kaolin clay has been considered (Hazzard et al. 2002; Delate 2003; Delate and McKern 2004). Kaolin (kaolinite) is a clay of fine, non-abrasive particles that disperse easily in water. Kaolin is sprayed on the plant foliage and forms a white, porous film which disrupts the insects' host finding abilities by changing visual, tactile, gustative or olfactory cues of the host plant (Glenn et al. 1999; Puterka et al. 2000; Puterka et al. 2005). The primary mechanism of action found was the repellence of insects from treated foliage (Glenn and Puterka 2005). The effects of kaolin depend on the insect species and are specific to the insect behavior and biology. Phytophagous insects feeding or ovipositing on plant tissues are highly susceptible to kaolin treatments (Knight et al. 2001).

Kaolin has, also, been shown to have beneficial effects on the growth of plants, especially under warm and dry conditions. These effects are attributed to the capacity of kaolin to reduce stress of plants exposed to excessive temperature by improving net photosynthesis (Erez and Glenn 2004). However, in temperate conditions with an abundance of water, carbon assimilation can be reduced since the optimal temperature for photosynthesis is not reached (Glenn et al. 2001a). Schupp et al. (2002) observed reduced

apple size and color with late applications of kaolin and Makus (2000) reported that kaolin delayed tomato fruit development. The label of the commercial formulation of kaolin, Surround<sup>®</sup>WP, specifies that pome fruit maturity may be delayed by 3 to 7 days especially in cool regions (PMRA 2006). However, to date, no studies have been carried out on the physiological effects of kaolin on cucurbit plants.

### **Hypothesis and objectives**

The hypothesis of this work is that kaolin can repel the SCB and control beetle infestations in field without negatively affecting cucumber growth. The objectives of this research are:

1. To determine the efficacy of kaolin particle-film in reducing SCB population, plant damage, bacterial wilt infection and cucumber yields compared to conventional practices under field conditions.
2. To evaluate the impact of kaolin coating on leaf gas exchange and growth of cucumber plants.
3. To investigate the behavior of SCB in contact with kaolin coated-plants.

## CHAPTER ONE

### Literature review

#### *Biology of the striped cucumber beetle*

The striped cucumber beetle (SCB; *Acalyma vittatum* Fabricius; Coleoptera: Chrysomelidae: Galerucinae: Luperini: Aulocophorina) belongs to the cucumber beetles group. Other cucumber beetles, also called diabroticite beetles, can also damage cultivated cucurbits, such as the northern corn rootworm, *Diabrotica barberi*, the spotted cucumber beetle, *Diabrotica undecimpunctata howardi* and the western corn rootworm, *Diabrotica virgifera virgifera*, but they appear later in the growing season in Québec and do not have as much economic impact as SCB.

In Canada, SCB has one generation per year, but in the U.S., depending on the length of the growing season, there can be up to three or more generations (Ellers-Kirk 1996). In Québec, there are two peaks of SCB over the season: the overwintering adults in the spring and the summer adults emerging from soil at the end of July (Duval 1994; Villeneuve and Couture 2004). SCB overwinter in the adult stage in plant debris and emerge when the daily mean temperature reaches 12°C (Radin and Drummond 1994b). In the spring, before the plantation of cultivated cucurbits, SCB can feed on pollen of different species, including plants from the genera *Ambrosia*, *Asclepias*, *Cucurbita*, *Daucus*, *Helianthus*, *Rosa*, *Solidago* and *Zea* (Houser and Baldus 1925; Gould 1944; Metcalf et al. 1998). However, SCB is completely dependent on cucurbits for successful reproduction and is more specific to cucurbits than other diabroticite beetles.

As soon as cucurbit seedlings emerge or are transplanted, SCB migrate into cucurbit fields. Despite its good flying abilities (over 800 meters; Duval 1994), SCB tend to aggregate in a large number on the same plant (Radin and Drummond 1994b). After mating, females lay their eggs in humid cracks in the topsoil, at an average depth of 5 cm (Necibi et al. 1992). The eggs are laid within a 12-15 cm diameter around the stem (Duval 1994). Mating and oviposition activities occur at temperatures ranging from 18 to 26°C with the minimum at 13 and 10°C, respectively (Radin and Drummond 1994b). According to Ellers-Kirk (1996), a female can lay 0 to 4 eggs per day for a total of 125 eggs in 80 days. Egg incubation takes five to nine days. Larval development has three

instars and is completed in 22 days at 27°C. Larvae feed on cucurbit roots and tunnel into the base of plant stems. Pupae develop for 6 to 7 days in the soil. The egg to adult cycle is completed in 31 days at 27°C (Ellers-Kirk and Fleischer 2006).

### ***Cucurbits and cucurbitacins***

Cultivated cucurbits all belong to different genera of the Cucurbitaceae family: cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), squash and pumpkin (*Cucurbita pepo* L., *Cucurbita moshata* Duch. and *Cucurbita maxima* Duch.) and watermelon (*Citrullus lanatus* (Thunb.) Matsum.& Nakai.). Cucurbits are annual crops grown as vines. Among cucurbits, cucumber is the main crop grown in Québec in 2005 (971 ha, 21 660 metric tons with a value of \$7 600 000 CAN), followed by pumpkin (627 ha, 14 628 tons and \$2 420 000 CAN) and squash (506 ha, 6804 tons and \$3 400 000 CAN) (Statistics Canada 2007). For this reason, we chose to conduct our experiments on cucumbers. Commercial slicing and pickling cucumber cultivars are predominantly gynecious, with only female flowers on the same plant, but some monoecious pollinizer seeds, plants with both male and female flowers on the same plant, are mixed in a proportion of 10-15% to ensure a good supply of pollen (Schultheis et al. 2007). Pollination of cucurbits relies mainly on gourd bees and honey bees (Wien 1997; Gingras et al. 1997, 1999).

Cultivated cucurbits are all attacked by the striped cucumber beetle but to differing degrees. Howe et al. (1972) observed that the SCB preferred the leaves of *Cucurbita maxima* followed by *Cucumis sativus* in an experiment comparing eight cucurbit species. SCB preferred wild cucurbits due to their greater amount of cucurbitacins in the foliage; among cultivated species, SCB preferred Hubbard squash (*Cucurbita maxima*) and zucchini (*Cucurbita pepo*) (Howe et al. 1976). In a study by McGrath and Shishkoff (2001), zucchini (*Cucurbita pepo*) and *C. maxima* were preferred to *C. moshata*, other winter and summer squash and gourd, and ultimately muskmelon, cucumber and watermelon. The preference of SCB for zucchini and *C. maxima* squash have been reported by several authors (Gould 1944; Wiseman et al. 1961; Ferguson et al. 1983; Elsey 1988; Hoffmann et al. 1996b; Reiners and Petzoldt 2006).

Cucurbits contain plant secondary metabolites called cucurbitacins. They are non-volatile, bitter, tetracyclic triterpenes, toxic to many vertebrates and invertebrates

(Metcalf and Rhodes 1990). Cucurbitacins are the bitterest compounds known in nature and can be detected by human at a concentration of 1 ppb (Metcalf et al. 1980). Cucurbitacins protect plants against herbivores. The repulsive effect of cucurbitacins has been proven for many herbivorous insects: *Phyllotreta nemorum*, *P. undulatas*, *P. tetrastigma*, *Phaedon cochliariae*, *P. cruciferae* and *Ceratoma trifurcata* (Metcalf and Rhodes 1990). There are more than 20 different cucurbitacins in the Cucurbitaceae family. Cucurbitacins B has the widest distribution among this plant family (Metcalf and Metcalf 1992). The cucumber (*Cucumis sativus* L.) is the only species rich in cucurbitacin C.

Cucurbitacins can be found in all parts of the plant. Fruit and roots of cucurbit plants have generally higher concentrations of cucurbitacins than do the leaves (Metcalf et al. 1982; Tallamy and Krischnik 1989). Quantities of cucurbitacins in the roots increase with the age of the plant (Metcalf and Lampman 1989). Cucurbitacin concentrations in fruits of cultivated *Cucurbita* species are generally low (Metcalf et al. 1982). Cucurbitacin content varies with leaf development, with young leaves having lower concentrations than older leaves in *Citrullus vulgaris* and *C. ecirrhosa* (Metcalf and Metcalf 1992). Cucurbitacin content in the cotyledons is not related to concentrations in other parts of the plant and can be higher or lower depending on species (Ferguson et al. 1983). In the case of *C. pepo*, cotyledons have higher levels of cucurbitacins than the true leaves. Cucurbitacins are often present in the flowers, as in the case of *Cucurbita maxima* (Andersen et Metcalf 1987). When a leaf is injured, cucurbitacin concentration increases in that leaf and adjacent leaves within a few hours (Tallamy 1985). Cucurbitacins content was higher in bitter wilted cucumbers than non-wilted or non-bitter (Haynes and Jones 1975).

### ***Plant-insect interactions***

Diabroticite beetles are able to sequester and metabolize the different types of cucurbitacins; it protects them from predators for whom cucurbitacins are toxic (Howe et al. 1976; Ferguson and Metcalf 1985). These species can consume cucurbitacins without appreciable fitness costs (Tallamy and Gorski 1997). This is explained by the narrow association between the diabroticites species and cucurbits which have coevolved (Metcalf and Lampman 1991; Metcalf and Metcalf 1992).

Cucurbitacins are kairomones for adult and larvae diabroticite beetles; they act as an arrestant for locomotion and as feeding stimulant (Metcalf et al. 1980). The cucurbitacin content of a plant is correlated with beetle feeding damage (Ferguson et al. 1983). Diabroticites have cucurbitacin receptors located in their maxillary palpi (Metcalf et al. 1980). *Diabrotica undecimpunctata howardi* can detect cucurbitacins at lower concentrations, than *D. u. undecimpunctata*, followed by *D. virgifera* and *Acalymma vittatum* (Metcalf et al. 1980; Tallamy et al. 1997). The feeding stimulant effect of cucurbitacins and the preference for high-content cucurbitacin plants decrease after continuous exposure of SCB to a cucurbitacin source (Tallamy and Gorski 1997; Smyth et al. 2002). This may be due to the insects having sequestered enough cucurbitacins to prevent predator attacks.

However, cucurbitacins which are not volatile are not recognized by the insect over any great distance; in fact they are perceived by the insect at distances of less than 1.5 mm (Howe et al. 1976). SCB are able to colonize cucurbitacin-free cucumber plots as rapidly as cucurbitacin-rich plants (Smyth 2002). SCB use other cues than cucurbitacins in finding their host plants. SCB are attracted by the color yellow and most of the flowers of the cultivated cucurbits are yellow (Andersen and Metcalf 1987; Hoffmann et al. 1996a). *Diabrotica* spp. have been shown to be sensitive to yellow-green and near-ultraviolet spectra (Agee et al. 1983). However, SCB are able to find *Cucurbita maxima* seedlings without visual or gustatory stimuli (Lewis et al. 1990). Adult SCB are attracted by host plant volatiles released by the cotyledons of cucurbits and by the male flower (Andersen and Metcalf 1986; Lewis et al. 1990). The mimic odor of *C. maxima* blossoms (1,2,4-trimethoxybenzene, indole and trans-cinnamaldehyde; TIC) has been reconstituted and can be used in baited traps (Lewis et al. 1990).

Andersen and Metcalf (1987) stated that visual characteristics of blossoms are important for host selection in combination with olfactory cues. High levels of volatiles would increase the arrival rate and the presence of cucurbitacin would decrease the departure rate. The study of the movement of adult SCB by Lawrence and Bach (1989) showed that SCB density is determined by the choice made in the initial colonization of the plot. Once installed, the SCB have a lower migration rate (24%) than the spotted cucumber beetle (38%). However, SCB migrate longer distances than the spotted



cucumber beetle. Moreover, SCB prefer plots not surrounded by non-host vegetation during initial colonization. Furthermore, Bach (1980a) stated that SCB number was more strongly related to total plot characteristics rather than individual plant characteristics. Bach (1980b) found that marked *A. vittatum* stayed in the same area after release into a cucumber monoculture, meaning that the natural habit of the beetle is not to migrate when a suitable host plant is available.

In parallel, aggregation behavior of the SCB was explained by an aggregation pheromone released by “male pioneers” feeding on plants and attracting both sexes (Smyth and Hoffmann 2002, 2003; Morris et al. 2005). Also, the feeding rate increased the response to the pheromone. The presence of cucurbitacin in the plants was not a prerequisite for the aggregation behavior (Smyth and Hoffmann 2002).

### ***Bacterial wilt***

SCB and the spotted cucumber beetle are the primarily vectors of bacterial wilt, *Erwinia tracheiphila*. Other cucumber beetles and insects that cause wounds can also disseminate the bacteria (Radin 1996). *Erwinia tracheiphila* has the ability to overwinter in the gut of the SCB and the spotted cucumber beetle (Garcia-Salazar and Gildow 2000; Garcia-Salazar et al. 2000). *Erwinia tracheiphila* can also overwinter on herbaceous weeds on which beetles feed and then spread the bacteria (Bassi 1982; Blua et al. 1994). However, this means of propagation is not important in nature (Mackiewicz et al. 1998). Plant infection occurs through the contact of the beetle frass or infected mouthparts with damaged foliage (Leach 1964; Yao et al. 1996). The rate of development of bacterial wilt is dependent on the amount of *E. tracheiphila* inoculated (Lukezic et al. 1996) and the age of the plant at inoculation (Radin 1996). The rate of wilt development is most rapid in young and succulent plants. Temperatures over 26°C speed up the development of the bacteria but at over 30°C, rate of development decreases (Jarvis 1994). Once inside the plant, the bacteria multiply and plug the vascular system, inhibiting translocation of water and nutrients causing wilting (Main and Walker 1971). The first symptoms of infection appear two to six days after inoculation (Main and Walker 1971; Watterson et al. 1972; Yao et al. 1996). At first, a single leaf is affected but wilt symptoms rapidly spread throughout the entire plant. Infected runners or plants die rapidly (Radin 1996). Infected fruit may be small, poorly shaped and wilted (Reiners and Petzoldt 2006). Signs of the

disease can be observed by the presence of viscous strings, white bacterial ooze, in the vascular system of a freshly cut stem (Jarvis 1994).

In a cucumber field, there is a positive correlation between cucumber beetle density and incidence of bacterial wilt (Yao et al. 1996). The control of bacterial wilt is entirely based on controlling insect vectors. Cucumber, melon and cantaloupe are more susceptible to the disease than squash and pumpkins while watermelon, *Citrullus lanatus*, is apparently unaffected (Fleischer and Kirk 1994; Radin 1996). However, bacterial wilt sensitivity is not always correlated with the cultivar's attractiveness to beetles (McGrath 2004). In trials conducted in New York State, zucchini was more attractive to beetles but less susceptible to bacterial wilt than cucumber. There is also variability in susceptibility among cultivars. Among cucumber varieties the pickling cucumber County Fair was less susceptible than the pickling cucumber Calypso and the slicing variety Dasher II (McGrath and Shishkoff 2001). Resistance to *E. tracheiphila* is known to be inherited as a dominant gene for cucumber but wilt-resistance germplasm was found to delay flowering and reduce yields (Staub and Peterson 1986).

### ***Striped cucumber beetle control methods***

The most common control method for the striped cucumber beetle is through the use of insecticides. Weekly applications of a contact insecticide can be sprayed when the SCB population emerges in the spring; however integrated pest management practices recommend the use of thresholds for intervention (Brust et al. 1996; MacIntyre-Allen et al. 2001b). Extension services in Québec (Réseau d'avertissement phytosanitaire; RAP) recommend monitoring SCB populations twice a week and applying insecticide when the threshold of 0.5-1 adult SCB per plant with less than five leaves or 3-5 adult SCB per plant with greater than five leaves is reached, or if there is fruit damage (Villeneuve and Couture 2004). In the Cucurbit IPM program of Cornell University, a threshold of 1 beetle per plant up to fourth leaf stage is used (Zitter et al. 2000) based on findings of Hoffmann et al. (2000). The extension vegetable program of the University of Massachusetts lowered the SCB threshold in pumpkin and squash to 0.5 beetles per plant from emergence to the fourth leaf stage due to recent widespread of bacterial wilt problems in those crops (Hazzard et al. 2002). The contact insecticides registered against SCB in Canada that can be used as part of the IPM program are: diazinon, endosulfan,

malathion and carbaryl, all from the organophosphate, organochlorine and carbamate families (Villeneuve and Couture 2006). One systemic insecticide, imidacloprid, is registered against SCB and has been successfully used in planting water and seed treatments (Pair 1997; Fleischer et al. 1998; MacIntyre-Allen et al. 2001a; Hazzard et al. 2002). This insecticide is used in smaller quantity (MacIntyre-Allen et al. 2001b) and is generally less harmful for the environment (Krohn and Hellpointner 2002). However, concerns about the possible toxicity of imidacloprid to honey bees, *Apis mellifera*, which act as cucurbit pollinators has been raised (Decourtye et al. 2003; Medrzycki et al. 2003; Halm et al. 2006). Integrated Pest Management (IPM) combines the use of chemical, biological and cultural strategies to reduce pest pressures. In the past, there has been a greater emphasis on the use of chemicals. However, increased insect resistance to pesticides and health concerns coupled with the growing interest in organic agriculture has raised the demand for alternative control methods. To develop a successful IPM strategy for SCB, a number of biological and cultural alternatives have been examined.

Among biological control methods, entomopathogenic nematodes (Reed et al. 1986; Eilers-Kirk et al. 2000), natural enemies and parasitism (Platt et al. 1999; Schroder and Athanas 2003; Gamez-Virues and Eben 2005), rhizobacteria (Zehnder et al. 1997), microbial metabolites (Reed and Reed 1986; Johnson et al. 1993) and the entomopathogenic fungus *Beauveria bassiana* (Gorjeltchan et al. 2002) have all been studied.

Many cultural methods such as alternative control like plastic mulch (Necibi et al. 1992), reflective mulch (Caldwell and Clarke 1999), floating row cover (Adams et al. 1990), vermicompost (Yardim et al. 2006), kairomonal baits (Brust and Foster 1995; Hoffmann et al. 1996a; Schroder et al. 2001) and trap crops (Radin and Drummond 1994a; Pair 1997; Cavanagh and Hazzard 2006) have all been tried. Recently the use of kaolin clay to control SCB has been considered (Hazzard et al. 2002; Delate 2003; Delate and McKern 2004).

### ***Kaolin particle film***

Kaolin (kaolinite) a white aluminosilicate clay, with fine and porous, non-abrasive particles disperses easily in water. This inert mineral is used in the paper, paint and plastic industries, depending on the processing and characteristics of the particles (size,

dimensions and brightness). It is also a component of drugs, toothpaste, cosmetics and alimentary products (Glenn and Puterka 2005).

Glenn and Puterka (2004) from USDA-ARS developed a formulation of kaolin particle film to protect crops from pests and diseases. According to Glenn and Puterka (2005), the mineral particle film must have the following properties: (1) chemically inert, (2) particle size under 2  $\mu\text{m}$ , (3) creation of a uniform film, (4) formation of a porous film allowing leaf gas exchange, (5) transmission of the photosynthetically active radiation (PAR) and reflection of the UV and the IR radiation, (6) interference with insect or pathogen behavior and (7) ability to be washed from the harvested products.

Kaolin particle film is available on the market as Surround<sup>®</sup> WP crop protectant. This commercial product is composed of hydrophilic kaolin particles mixed with an oil-based spreader-sticker (Glenn and Puterka 2005). This product is registered on the Organic Materials Review Institute (OMRI) list for use in organic agriculture in the U.S. and listed in the permitted products for organic production in Québec (CAAQ 2007). In addition to insect control, kaolin has been tested against fungal and bacterial diseases (Glenn et al. 1999, 2001b; Puterka et al. 2000; Lalancette et al. 2005) and for other horticultural benefits such as the protection of fruit against sunburn and enhancement of fruit color (Elkins et al. 2001; Glenn et al. 2001a, 2002, 2003, 2005; Schupp et al. 2002; LeGrange et al. 2004; Melgarejo et al. 2004; Wünsche et al. 2004; Gindaba and Wand 2005; Wand et al. 2006) or resistance to freezing (Wisniewski et al. 2002).

### ***Insect control with kaolin***

Kaolin was first tested on perennial crops such as fruit trees due to the increasing incidence of insect resistance with repeated insecticide applications (Glenn et al. 1999).

Kaolin has been shown to be effective against lepidopterans such as codling moth, *Cydia pomonella* (Puterka et al. 2000; Unruh et al. 2000; Friedrich et al. 2003), oblique banded leafroller, *Choristoneura rosaceana* (Knigh et al. 2000; Sackett et al. 2005), oriental fruit moth, *Grapholita molesta* (Lalancette et al. 2005), beet armyworm, *Spodoptera exigua* (Showler 2003), fruit tree leafroller, *Archips argyrospilla* (Knigh et al. 2001), diamondback moth, *Plutella xylostella* (Barker et al. 2006), undetermined species of leafrollers and pink bollworm, *Pectinophora gossypiella* (Sisterson et al. 2003).

It also has been shown to be effective against the psyllids pear psylla, *Cacopsylla pyricola* and *C. pyri* (Glenn et al. 1999; Puterka et al. 2000; Pasqualini et al. 2002; Daniel et al. 2005; Puterka et al. 2005). In 2003, over 50% of the U.S. pear growers were using Surround<sup>®</sup> WP to control pear psylla that had become resistant to most insecticides (Glenn and Puterka 2005). Other homopterans such as the glassy-winged sharpshooter, *Homalodisca coagulata* (Puterka et al. 2003; Tubajika et al. 2007), unspecified species of cicadellids (Showler and Setamou 2004) and the pistachio psyllid, *Agonoscena targionii* (Saour 2005) were controlled by kaolin in addition to the reduction of the beet curly top virus (BCTV) transmitted by the beet leafhopper in pepper (*Circulifer tenellus*; Creamer et al. 2005). While Glenn et al. (1999) found that kaolin was effective against the potato leafhopper *Empoasca fabae* in potato, Maletta et al. (2006) did not. However, they found it to be effective against the same insect in eggplant (Maletta et al. 2004). In the Hemiptera order, the tarnished plant bugs *Lygus lineolaris* and the stinkbugs *Acrosternum hilare*, *Euschistus servus* and *E. tristigmus* were controlled by kaolin in a peach crop (Lalancette et al. 2005).

In the Diptera order, the apple maggot fly *Rhagoletis pomonella* (Puterka et al. 2000; Garcia et al. 2004), the Mediterranean fruit fly *Ceratitis capitata* (Mazor and Erez 2004), the olive fruit fly *Bactrocera oleae* (Saour and Makee 2004), the blueberry maggot, *Rhagoletis mendax* (Liburd et al. 2003) and dipterans from drosophilidae, cecidomyiidae and muscidae families in cotton (Showler and Setamou 2004) were all controlled by applications of kaolin. Kaolin was effective in controlling flower thrips (*Frankliniella* spp.; Thysanoptera: Thripidae) in blueberry plants (Spiers et al. 2004) but not in peanuts (Wilson et al. 2004). Thrips damage (*Scirtothrips aurantii*) was also reduced by kaolin on mango fruits (Joubert et al. 2004).

In the case of coleopterans, the boll weevil *Anthonomus grandis* in cotton (Showler 2002a) was effectively controlled by kaolin in both small and large plots whereas root weevil *Diaprepes abbreviatus* (Lapointe 2000) was deterred from kaolin coated citrus in the laboratory but its larvae were not reduced on citrus tree roots in the field (Lapointe et al. 2006). The number of mango weevils, *Sternonchetus mangifera*, was reduced in mango groves but fruit damage was not significantly lower than untreated trees (Joubert et al. 2004). Plum curculio *Conotrachelus nenuphar* (Puterka et al. 2000;

Lalancette et al. 2005) and Japanese beetle (*Popillia japonica*) were controlled in apple and pear orchards (Lalancette et al. 2005). Kaolin was not effective in controlling flea beetles (Coleoptera: Chrysomelidae: Alticinae; various species) in komatsuna (*Brassica rapa*; Andersen et al. 2006) but was effective in eggplant (Maletta et al. 2004).

In general, insects controlled by kaolin in the laboratory were also controlled in the field, with the exceptions of whiteflies, aphids and two-spotted spider mites (Knight et al. 2001; Cottrell et al. 2002; Liang and Liu 2002; Poprawski and Puterka 2002; Garzo et al. 2003; Showler and Setamou 2004; Wyss and Daniel 2004; Burgel et al. 2005; Glenn and Puterka 2005; Eigenbrode et al. 2006). Under field conditions, it is difficult to insure complete coverage with the film and these insects simply move to non-treated areas.

In some cases, kaolin particle film can increase pest pressures due to its effects on beneficial insects. A study on secondary pests and beneficials of apples (Knight et al. 2001), revealed that the leafminer, *Phyllonorycter elmaella*, was significantly higher in kaolin treated trees because the proportion of parasitized leafminers was reduced. In kaolin-treated apple trees, a secondary pest San Jose scale, *Quadraspidiotus perniciosus*, caused the main damage and rosy apple aphid *Dysaphis plantaginae* was increased. The number of spiders and generalist predators was also reduced. In pear orchards, kaolin increased red mites, *Panonychus ulmi* (Lalancette et al. 2005). In a study on pecan trees carried out by Lombardini et al. (2005), the main pest of pecans, the pecan borer (*Acrobasis nuxvorella*), increased in kaolin-treated trees while the eggs of chrysops, *Chrysoperla camea*, a predator, decreased. However, adult chrysops and other natural enemies, such as lady bugs and spiders, were not affected. Mango scale, *Aulacapsis tubercularis*, and the secondary pest long-tailed mealybug, *Pseudococcus longispinus*, were increased with kaolin applications in mango groves (Joubert et al. 2004). Numbers of cotton aphids, *Aphis gossypii*, were increased by kaolin treatment and beneficial insects from many families were reduced in cotton (Showler and Sétamou 2004).

However, since the positive effects of the use of kaolin greatly outnumber the negative effects, Surround® WP has been registered in the U.S. for all main crop groups. In Canada, Surround® WP is registered in pome fruits, grapes and nut trees against pear psylla, oblique banded leafroller, tarnished plant bug, apple maggot, leafhoppers, plum curculio, codling moth, oriental fruit moth, walnut and hazelnut leafrollers, walnut husk

fly, butternut curculio and since August 2005, against cucumber beetles in cucurbits (PMRA 2006).

### ***Mode of action of kaolin against insect pest***

The initial hypothesis concerning kaolin's ability to control insect pests was that kaolin disrupted the insects' host finding abilities by changing visual, tactile, gustative or olfactory cues of the host plant (Glenn et al. 1999; Puterka et al. 2000; Puterka et al. 2005). The primary mode of action found was the repellence of adults from treated foliage (Glenn and Puterka 2005). Particle attachment to insect body parts have been also identified as a possible effect (Glenn et al. 1999; Puterka et al. 2005). There was reduced settlement and oviposition of insects on kaolin-coated plants (Glenn et al. 1999; Lapointe 2000; Puterka et al. 2000; Unruh et al. 2000; Showler 2002a; Puterka et al. 2003; Sisterson et al. 2003; Showler 2003; Sackett et al. 2005). Showler (2002a) and Barker et al. (2006) demonstrated that kaolin affected the insect at a distance rather than being limited to direct contact. A reduced survival of adult and larvae was recorded on kaolin-treated foliage (Knight et al. 2000; Unruh et al. 2000; Cottrell et al. 2002; Showler 2003; Puterka et al. 2005; Sackett et al. 2005). In addition, there was a reduction in the mating of lepidopterans exposed to kaolin (Knight et al. 2000; Puterka et al. 2005). The effects of kaolin depend on the insect species and are specific to the insect behavior and biology. Knight et al. (2001) generalized that efficacy of kaolin depended on the feeding habit of each insect species. Phytophagous insects feeding or ovipositing on plant tissues were highly susceptible to kaolin treatments. For insect species such as aphids, leafminers and leafrollers that feed on young leaves or on protected plant parts like shelters, the efficacy of kaolin was variable.

### ***Kaolin against the striped cucumber beetle***

Very few studies have been conducted to prove the efficacy of kaolin against cucumber beetles in cucurbits. Kirkland (2003) performed a study in California at the request of Engelhard Corp., the manufacturer of Surround<sup>®</sup>WP. This study targeted the western spotted cucumber beetle, *Diabrotica undecimpunctata*, and the western striped cucumber beetle, *Acalymma trivittata*, comparing kaolin (Surround<sup>®</sup>WP), insecticide (Sevin<sup>®</sup>XLR PLUS) with an untreated control with cucumber cv. Poinsett 76. Kaolin was

applied weekly, from the fourth leaf stage to fruit set, for a total of five applications. Maximum beetle density in control treatment was 9.3 beetles per plant, 1.8 in insecticide and 1.5 in kaolin. Twenty-six percent of the leaves were damaged in control, 1.3% in insecticide and 2.5% in kaolin treatments. There was no difference in yields among treatments but kaolin reduced by 10% the number of sunscalded fruit. Bacterial wilt was not present on the experimental site and there was no precipitation for the duration of the experiment therefore the kaolin was not washed off. The conditions of this trial are very different from what we observe in eastern Canada with a massive migration of beetles at the youngest stages of cucurbit development coupled with severe incidence of bacterial wilt in some areas and precipitation during the growing season.

Researchers in the northeastern U.S. tested kaolin (Surround<sup>®</sup>WP) as a mean of controlling SCB in cucurbits. However, results have been variable depending on year and beetle populations. Indeed, in 2001, Hazzard et al. (2002) observed a reduction in leaf damage on kaolin-treated pumpkins, a decrease of bacterial wilt infected plants, an equivalent yield to other treatments for transplants and a greater yield for kaolin-treated direct seeded pumpkins. However, the following year, damage and bacterial wilt severity were not statistically different between kaolin and control treatments for transplants and direct seeded plants and yields were not different from the control. Delate (2003) and Delate and McKern (2004) did not observe reductions of squash bug (*Anasa tristis*), squash borer (*Melittia cucurbitae*), beneficials, nor bacterial wilt in a squash cv. Zenith treated twice a week with kaolin (Surround<sup>®</sup>WP). However, the yields of the kaolin-treated plants were 17% higher than that of the control.

### ***Effect of kaolin on leaf gas exchange and plant productivity***

The kaolin formulation developed by Engelhard Corp. and the USDA-ARS comes from purified and heat-activated kaolin particles forming a porous film which does not block stomata opening (Glenn and Puterka 2005). In addition, the optic properties of this formulation allow photosynthetically active radiation (PAR; 400-700 nm) to pass through the film and reach the plant surface and chloroplasts with a minimal PAR light reflection of 10% (Glenn et al. 1999).

Kaolin has been shown to have beneficial effects on the growth of plants, especially under warm and dry conditions. These effects are attributed to the capacity of



kaolin to reduce stress of plants exposed to excessive temperature by improving net photosynthesis (Erez and Glenn 2004). Glenn et al. (1999) found no reduction in photosynthetic activity of apple, peach and pear leaves with particle levels up to 3000  $\mu\text{g}/\text{cm}^2$  of leaf surface. Glenn et al. (2001a) observed that kaolin-treated apple trees had a lower canopy temperature however single-leaf stomatal conductance, transpiration and carbon assimilation, as a measure of photosynthesis, were increased. In seven of the eight apple trials, yield or fruit weight was increased when air temperature was greater than 30°C. However, in temperate conditions with an abundance of water, carbon assimilation was reduced since the optimal temperature for photosynthesis was not reached. Glenn et al. (2001a) come to the conclusion that kaolin application on plants under heat stress brought more advantages than the reduction of 10% of PAR because it reduced leaf temperature and increased net photosynthesis efficacy.

Furthermore, Glenn et al. (2003) observed an increase in yield and fruit quality on kaolin-treated apple trees caused by an increase in whole-tree carbon assimilation under high temperature conditions. However, water use efficiency was reduced due to an increase in stomatal conductance associated with reduced leaf temperature, which increased transpiration more than carbon assimilation. Puterka et al. (2000) also found an increase in yield with kaolin use in pear production in West Virginia. Jifon and Syvertsen (2003) found that, under high temperature and radiation, kaolin application on grapefruit leaves (*Citrus paradisi* L.) increased single-leaf stomatal conductance, carbon assimilation and water use efficiency without affecting intercellular CO<sub>2</sub> concentration and leaf transpiration. However, an increase in water use efficiency in individual leaves did not result in an increase at the whole-tree level.

Lapointe et al. (2006) measured greater growth of kaolin-treated citrus trees in Florida and precocious fruit production. A pre-fruit (50% bloom) single application of kaolin on blueberry plants increased plant growth and yield but reduced berry size (Spiers et al. 2003). On ‘McIntosh’ apple in New England orchards, preliminary studies showed that kaolin combined with fungicides applications had a beneficial impact on fruit weight and color but no effect on yield (Garcia et al. 2004). Weekly kaolin applications to cotton plants did not result in differences in either water potential or cotton yields. Amino-acids profiles of kaolin-treated cotton plants suggest that kaolin does not induce shade stress

and that kaolin's reflectivity may heighten light reception (Showler 2002b). Creamer et al. (2005), in southern New Mexico, found a reduction in water stress of kaolin-treated peppers, an increase in photosynthetic activity and chlorophyll. However, the yield did not differ from that of the control.

Sugar et al. (2005) in Oregon found no difference in the growth of pear trees with full-season kaolin application. On pecan trees (*Carya illinoensis* 'Pawnee'), kaolin reduced the leaf temperature but had no effect on leaf carbon assimilation, stomatal conductance, yield or the quality of the nuts (Lombardini et al. 2005). Russo and Diaz-Pérez (2005) found no difference in carbon assimilation, stomatal conductance, transpiration, leaf temperature and yields when kaolin was applied to peppers (*Capsicum annuum* L.). Nakano and Uehara (1996) observed an increase in water loss of kaolin treated tomato plants without influencing the transpiration stream via the stomata. They concluded that kaolin enhanced transpiration through the cuticle of the tomato leaf and fruit. Tworowski et al. (2002) measured growth of bean plants after eight consecutive applications of kaolin and found no effect on carbon assimilation at different light intensities, but reductions in stomatal conductance, transpiration and root dry weight, an increase in shoot-to-root ratio and no effect on leaf area.

On the other hand, LeGrange et al. (2004) found a reduction in carbon assimilation of kaolin-treated apple leaves under light-limited conditions and mild temperatures. This is presumably due to an increase in light reflection and a decrease in light available to the chloroplasts. Wünsche et al. (2004) observed a decrease in leaf carbon assimilation of kaolin-treated apple leaves without any effect on stomatal conductance and transpiration. They measured an increase in leaf light reflectance of 20%. At high light intensity, kaolin-treated leaves maintained their photosynthetic abilities while control leaves reached their photosynthetic maximum at lower light intensity. However, the decrease of carbon assimilation was not observed at the whole-tree level, suggesting that kaolin can improve light distribution within the canopy. Rosati et al. (2006) confirmed this hypothesis in a study showing that kaolin reduced light-saturated CO<sub>2</sub> assimilation rate without any effect on stomatal conductance and increased intercellular CO<sub>2</sub> concentration in walnut and almond leaves. The reduction in carbon assimilation of kaolin-treated trees was negligible compared with the effect of water

stress. Subsequently, they modeled the photosynthetic response of kaolin-treated leaves and found a reduction in PAR of 37% on the almond tree leaves (Rosati et al. 2006). The effect on single leaves did not translate to the whole canopy as there was no reduction in global carbon assimilation (Rosati et al. 2007). In both these studies (Wünsche et al. 2004; Rosati et al. 2007), kaolin film reduced carbon assimilation at the leaf-level but improved light distribution in the tree canopy increasing carbon assimilation at the tree-level, confirming the negligible or positive effect of kaolin on yields.

There have been relatively few studies which reported negative effects of kaolin on plant growth and yields. Schupp et al. (2002) observed reduced apple size and color with late applications of kaolin and Makus (2000) reported that kaolin delayed tomato fruit development without affecting leaf temperature and transpiration. The Surround®WP label specifies that pome fruit maturity may be delayed by 3 to 7 days especially in cool regions (PMRA 2006).

## CHAPTER TWO

### **Evaluation of kaolin for the control of the striped cucumber beetle, *Acalymma vittatum* (Coleoptera: Chrysomelidae) attacking cucumber**

#### **Introduction**

Striped cucumber beetle (SCB), *Acalymma vittatum* (Fabricius) (Coleoptera: Chrysomelidae), is the main pest of cucurbits in northeastern America. It is an herbivorous cucurbit specialist. It feeds on cucurbit foliage and transmits bacterial wilt, *Erwinia tracheiphila* (Smith). SCB is univoltine under Québec conditions and two peaks of beetles are observed: the overwintering generation appears in the spring and the summer beetles emerge at the end of July and overwinter as adult (Duval 1994). Seedling mortality is the primary cause of yield reduction. Early defoliation is particularly critical in the growth and development of the plant. Early summer squash defoliation by beetles reduces the number of flowers and early season yield (Brewer et al. 1987). Once plants are infected by bacterial wilt nothing can be done to avoid the expression of wilt symptoms. Bacteria plug the vascular system and inhibit translocation of water and nutrients (Jarvis 1994). Infected runners or plants wilt and die rapidly (Radin 1996). Infected fruit may be small, poorly shaped and wilted (Reiners and Petzoldt 2006). The incidence of bacterial wilt is highly correlated with cucumber beetle density (Yao et al. 1996). Later in the season, the summer generation of SCB feed on fruits which, then, become unmarketable.

Pesticide reduction is of primary concern in vegetable production and there is a need for alternative pest control methods. I investigated a new method for the control of the striped cucumber beetle: kaolin clay. Kaolin is white, non abrasive and inert aluminosilicate clay marketed as Surround® WP. The product is mixed with water and sprayed on the foliage. The first application should be carried out to protect the seedlings prior to the migration of beetles in the cucumber field. Repeated applications are necessary to maintain kaolin coverage on newly developed leaves and after rain. The film acts as repellent interfering with insect host finding abilities. The efficacy of the product has been reported for a range of insects in a variety of crops. However, the exact

mechanisms of action are not known. Kaolin has been shown to be effective against fruit tree pests such as codling moth, *Cydia pomonella* (Puterka et al. 2000; Unruh et al. 2000; Friedrich et al. 2003), oblique banded leafroller, *Choristoneura rosaceana* (Knighth et al. 2000; Sackett et al. 2005), oriental fruit moth, *Grapholita molesta*, (Lalancette et al. 2005), pear psylla, *Cacopsylla pyricola* (Glenn et al. 1999; Puterka et al. 2000; Puterka et al. 2005), apple maggot fly *Rhagoletis pomonella* (Puterka et al. 2000; Garcia et al. 2004), plum curculio *Conotrachelus nenuphar* (Puterka et al. 2000; Lalancette et al. 2005) and glassy-winged sharpshooter *Homalodisca coagulata* in grapes (Puterka et al. 2003; Tubajika et al. 2007). In the case of coleopterans, kaolin was effective against the boll weevil *Anthonomus grandis* in cotton (Showler 2002a) but was not effective against flea beetles (Coleoptera: Chrysomelidae: Alticinae; various species) in komatsuna (*Brassica rapa*; Andersen et al. 2006) but was effective against these insects in eggplant (Maletta et al. 2004). To test the efficacy of kaolin against the striped cucumber beetle (*Acalymma vittatum*), I conducted field experiments with cucumbers (*Cucumis sativus* L.).

## **Material and Methods**

### ***Field preparation and experimental layout***

Field experiments were conducted during the summers of 2005 and 2006 at the Horticulture Research Center, Macdonald Campus, McGill University, Sainte-Anne-de-Bellevue, Québec (lat. 45° 26'N, long. 73° 56'W). The experimental sites were Gleyed Eluviated Eutric Brunisols which were fall-ploughed and spring-harrowed. The 2005 and 2006 fields had pHs of 7.1 and 7.4, and 7.1% and 5.0 % organic matter, respectively. Soils test indicated high nutrient levels (Lalande, June 2005) and the rate of fertilization was adjusted according. A 20N-8.7P-16.6K fertilizer was broadcasted at 200 kg/ha and disked in before planting which corresponds to half the provincial recommendations for nitrogen (Centre de référence en agriculture et agroalimentaire du Québec, 2003). Nitrogen input was complemented via fertigation throughout the summer. A black mulch (0.025 mm thick, 1.2 m wide; Climagro, Plastitech, St-Remi, Québec) and drip irrigation line (T-Tape, 16 mm diameter, 30 cm emitter spacing, 250 L/h/100m; T-Systems Inc., San Diego, CA, USA) was installed one week prior to seeding. In 2005, the experimental design was a completely randomized design with four replicates. For the 2006 field

season, this was modified to a randomized complete block design with 4 blocks because of the soil heterogeneity in the field. In 2005, plots consisted of six 8 m rows separated by a 2 m of bare ground. The outer two rows on each side of the plot acted as borders and data were taken only on 6 meters in the 2 inner rows. In 2006, based on observations of beetle movement in the 2005 trial, the plots were redesigned. Plots had four 6m rows with a 12 meter barley border (cv. Viviane, 108 kg/ha) between plots to improve plot independence (Lawrence and Bach 1989). Data were collected from the 2 inner rows. Plants were spaced 0.30 m on the row and rows were spaced at 1.5 m (2005) or 1.75 m (2006).

Cucumbers (*Cucumis sativus* L. cv. Speedway; Semences B.C., Laval, Québec), were manually seeded into the mulch with three seeds per hole June 7, 2005 and May 29, 2006. Speedway is a hybrid cultivar and was chosen because of its precocity (55 days from seeding to harvest), its resistance to a large number of diseases (downy mildew, anthracnose, powdery mildew, cucumber mosaic virus; Reiners and Petzoldt 2006) and its widespread use among Québec growers. The seeds were coated with a fungicide (thiram; Bayer CropScience, Research Triangle Park, NC, USA). After emergence, seedlings were thinned to one per hole. Fertilization was applied weekly through the drip irrigation system, the first 3 weeks with 150mg/L<sup>-1</sup> (ppm) of 20N-8.7P-16.6K followed by 4 weeks of 400 mg/L<sup>-1</sup> (ppm) of 15N-0P-0K-13.7Ca (Plant Products Company Ltd., Brampton, Ontario) for a total of 73 kg of nitrogen/ha per season. Additional irrigation was applied through the drip system as required. Weed control between the mulched rows was performed mechanically and no pest or disease control was used except for the experimental treatments.

### ***Experimental treatments***

Two striped cucumber beetle control treatments (insecticide and kaolin) were compared with an untreated control. For the insecticide and kaolin applications, a 16-liter pressurized backpack sprayer was used (ROSY; Di Martino spa (DM), Mussolente, Italy). The insecticide carbaryl was used at 1.1 L/ha of active ingredients (a.i.) (2.5 L/ha; Sevin<sup>®</sup> XLR PLUS; Bayer CropScience, Research Triangle Park, NC, USA). To determine when to spray the insecticide, we used either the Réseau d'avertissement phytosanitaire (RAP) threshold or commercial practice. RAP threshold is 0.5 to 1 adult SCB per plant when a

plant has less than 5 leaves and 3 to 5 adult SCB per plant for plants over five leaves or if fruit damage is present. However, in standard commercial practice producers often apply weekly applications of insecticide or as soon as they see an increase in the number of beetles. In 2005, since the inherent SCB population was low, we sprayed following the commercial practice at the first increase of beetle population in the spring and only one spray was necessary (July 2). In 2006, the insecticide was applied at the second leaf stage (June 19) and at the first flower stage (July 4) when the RAP threshold had been reached. Kaolin was applied at 23.75 kg/ha a.i. (47.5 g/L a.i., 50g/L of Surround<sup>®</sup>WP; Engelhard corp., Iselin, NJ, USA) based on the manufacturer's recommendation with the two first applications been given 3 days apart and re-application every 5 to 7 days and after heavy rain to maintain coverage on the foliage for a maximum of 5 applications. Kaolin was applied 4 times in 2005 at the cotyledon (June 20), first (June 23), third (June 30) and eighth leaf stages (July 10). In 2006, five applications were made at the cotyledon (June 8), first (June 12), second (June 19), fourth (June 23) and sixth leaf stages (June 29).

Due to the low inherent beetle pressure experienced in 2005, it was decided to increase the natural population by adding SCB to each plot. Therefore, adult *Acalymma vittatum* were collected between June 5 and June 7 2006 from organic cucurbit fields in the area (Senneville, Québec) and maintained at 4°C in containers equipped with a water-saturated cotton wick designed by Teshler et al. (2004) until their release on June 12. The SCB were counted and an equal numbers of beetles placed in each container. Containers were positioned at 4:30 p.m. in the center of each experimental plot and opened to release the beetles on June 12 when the cucumbers were at the first-leaf stage. This release time was chosen to prevent an increase in temperature in the containers following the methodology of Dernovici et al. (2006). In the kaolin treatment, the kaolin had been applied prior to the release of the insects. Eighty beetles were released in each plot to give a corresponding rate of 1 SCB per plant.

### ***Data collection***

Monitoring of insects and diseases was done twice a week from June 16 to July 21, 2005 and June 12 to July 13, 2006 and then weekly from the start of harvesting (August 2 to August 23, 2005 and July 19 to August 8, 2006). Ten plants were chosen randomly for each sampling date in the 2 inner rows of each plot and carefully inspected

for adult SCB. When plants had reached the fifth leaf stage, five leaves and three flowers were randomly chosen on 10 plants per plot for the insect counts. Counts were done in the morning between 8 and 11 a.m. and included beetles on the plant, on the soil surface near the stem and on the mulch under the sampled plant. Simultaneously, plants evaluated for insect number were also monitored for leaf damage based on the total number of leaves present between the appearance of the first true leaf to flowering. In 2005, a 0 to 4 scale was used for damage evaluation (0 = no damage, 1= 1-25%, 2= 26-50%, 3= 51-75 % and 4= 76-100 % of the leaf area defoliated by SCB) following Hazzard et al. (2002). The scale used in 2005 was not precise enough and was modified in 2006 to better define damage at the low end of the range. The scale used in 2006 was 0 to 5 scale (0 = no damage, 1= 1-10 %, 2=11-25%, 3= 26-50%, 4= 51-75%, 5= 76-100 % of the leaf area defoliated by SCB) (adapted from Brewer et al. 1987). Defoliation classes were converted to means of 0, 5.5, 18, 38, 63 and 88% and weighted averages of each class recorded.

Bacterial wilt infection was assessed three times in 2005 (July 27, August 9 and August 23) with the number of plants infected or dead noted over the total number of plants in the two middle rows. Bacterial wilt was monitored weekly in 2006 and disease severity was evaluated with the following Hazzard et al. (2002) scale: 0= absence, 1= <20% wilting, 2= 20 to 80% wilting, 3= 80% wilting, 4= dead. Plants infected by bacterial wilt were flagged and the evolution of the disease followed. In addition, plants killed by feeding damage were also flagged.

To confirm that the disease was indeed bacterial wilt, plant samples were sent to the provincial diagnostic laboratory (Laboratoire de diagnostic en phytoprotection du MAPAQ) for confirmation of the disease both years.

Cucumbers were harvested for 4 weeks for a total of 11 times in 2005 (July 25 to August 19) and 10 times in 2006 (July 17 to August 9). Cucumbers were classified according to an industry grading system based on the diameter and length of the fruits (Villeneuve, pers. comm. 2005). Marketable yields were composed of three classes, large (diameter 57-70 mm), super select (diameter 44-57 mm; length >152 mm) and select cucumbers (diameter < 44 mm; length >152 mm) with super select being the most desirable. The Canadian Food Inspection Agency (CFIA 2004) states that marketable field or slicing cucumbers must be green on 85% of the surface, well-shaped, free of



sunscald, scars, disease and broken skin. In this trial, unmarketable cucumbers were divided into three categories: damaged by SCB (scarring >5% of the surface), color defects and other (not straight, diseased, broken skin, other scars).

### ***Statistical analysis***

SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses with a probability level of 5%. Numbers of SCB per plant were square root transformed and each date analyzed separately with Tukey multiple comparison tests (GLM; SAS Institute inc.). The percentage of plants damaged by SCB, infected by bacterial wilt and dead were analyzed with a binomial model ( $r = \text{number of plants damaged, infected or dead per plot} / N = \text{total number of plants sampled or that emerged per plot}$ ; GENMOD; SAS Institute Inc.). Yields shown are the number of cucumbers harvested per plot divided by the number of plants that emerged in that plot; plant mortality was not taken into account. Mean number of cucumber fruits per plant were compared with Tukey multiple comparison tests. Due to the differences in both climate and experimental design, the results for the two years could not be pooled and are presented separately.

## **Results**

### **2005-season**

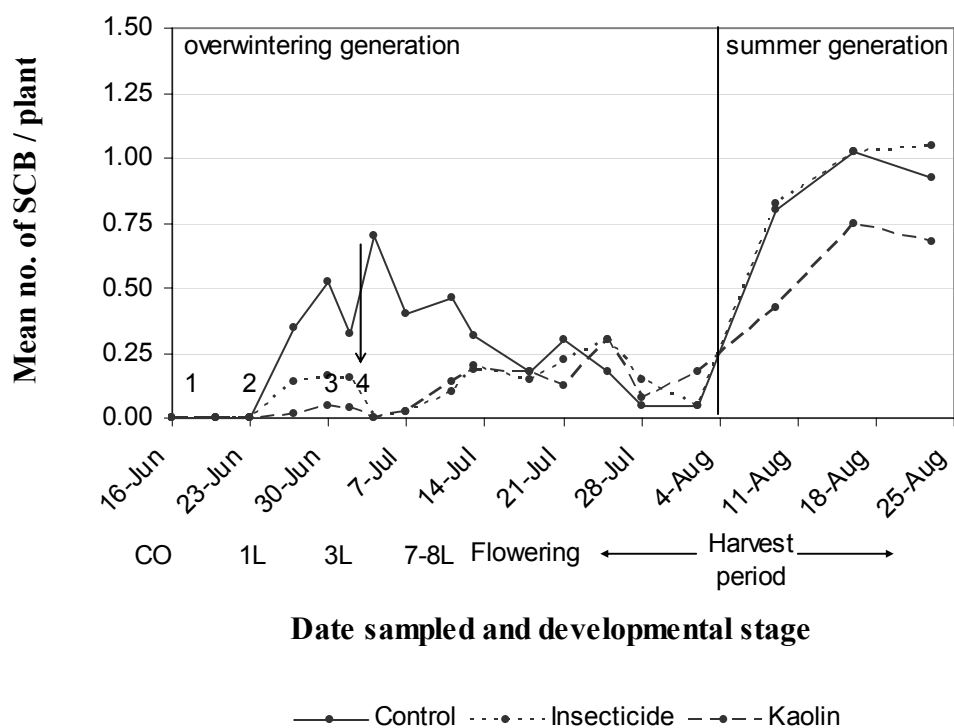
#### ***SCB populations, damage and bacterial wilt incidence***

The 2005 season was characterized by a cold spring with a frost on May 12 that delayed seeding, followed by a very hot wet summer with both temperature and precipitation values above the 29 year average (Appendices 1 and 2). SCB were already present in an adjacent melon field before the cucumber seedlings emerged. They colonized the experimental plots late, around June 24, when the cucumber plants were at the first leaf stage. No damage or death was observed at the cotyledonary and first leaf stage.

The overall SCB pressure was low. The overwintering generation of beetles in the control treatment reached a maximum 0.7 SCB per plant and the threshold for insecticide application was 0.5 to 1 beetle per plant. The overall SCB summer generation was lower

than the RAP thresholds. SCB summer generation reached 1.05 SCB per plant and threshold for this generation was 3 to 5 beetles per plant (Figure 2.1). Nevertheless, insecticide was applied at the fourth leaf stage when number of beetles in the insecticide plots reached 0.15 beetles per plant. The number of overwintering beetles was smaller than that of the summer generation which arrived in the first week of August. The number of SCB per plant in the control plots was significantly higher than those in the kaolin treatment for the three weeks after beetle emergence prior to the flowering stage (Figure 2.1 and Table 2.1). After the insecticide application, numbers of beetles were significantly reduced up to the flowering stage in the insecticide plots but continued to increase in control plots. This increase may be due in part to the movement of surviving beetles from the insecticide to control plots. However, beetle numbers in kaolin plots did not rise after the insecticide application indicating that beetles did not move to kaolin plots. The numbers of SCB in the insecticide plots were similar to those of the kaolin treatment throughout the 2005 growing season (Table 2.1). After July 13, there was no significant difference in SCB number among the three treatments, with the population decreasing over time until the arrival of the summer generation. The number of beetles in the kaolin treatment was lower but not significantly than that of the insecticide and the control treatments after the summer generation had emerged (August 9<sup>th</sup>). The seasonal mean number of beetles per plant (mean of 13 dates) was not significantly different in the kaolin ( $0.19 \pm 0.04$ ) and the insecticide treatment ( $0.26 \pm 0.05$ ) but those two were significantly lower than control ( $0.41 \pm 0.04$ ) according to the Tukey-Kramer test on square root transformed data.

Leaf damage was assessed at the youngest developmental stages, from cotyledon to flowering (Figure 2.2). Fewer plants were damaged in the kaolin compared with the insecticide treatment over this period. Differences were significant from the third to fifth-leaf stages (June 30 to July 4). The insecticide treatment showed significantly fewer damaged plants than the control from the fourth to seventh-leaf stages (July 2 to July 7). However, it was impossible to detect differences in the severity of damage among treatments because of the scale used as all plants had defoliation levels less than 25% of their total leaf area and after flowering stage all the plants were in category 1 (1-25%), showing at least 1% injury on the total leaf area.



**Figure 2.1.** Effect of insecticide and kaolin on the population of adult striped cucumber beetles (SCB; *Acalymma vittatum*) on cucumber plants (cv. Speedway) grown in south western Québec in 2005.

The developmental stage abbreviations are cotyledons (CO), first leaf (1L), third leaf (3L) and seventh to eighth leaf stage (7-8L). The arrow indicates the date of insecticide application and the numbers indicate dates of kaolin applications (1 to 4).

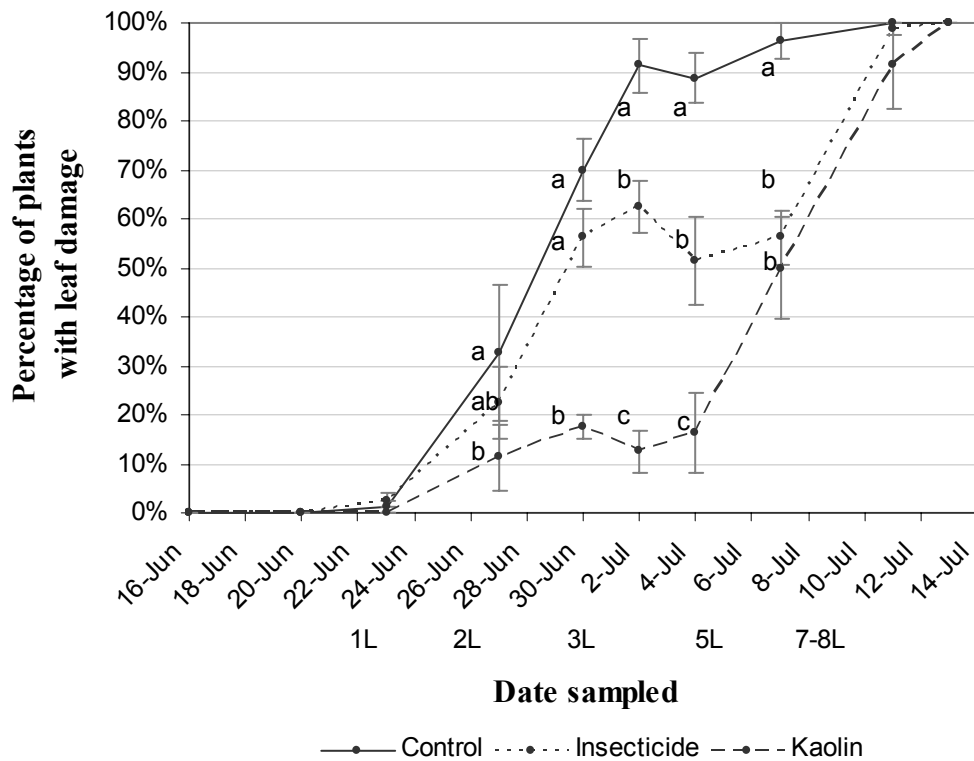
**Table 2.1.** Effect of insecticide and kaolin on the number of striped cucumber beetles (SCB; *Acalymma vittatum*) per cucumber plant in 2005 (mean  $\pm$  standard error).

Treatment	Mean no. SCB / plant							
	27-Jun	30-Jun	4-Jul	7-Jul	11-Jul	13-Jul	18-Jul	
	2L	3L	5L	7-8L	Flowering	Flowering	Flowering	
<b>Control</b>	0.35 $\pm$ 0.14 a	0.53 $\pm$ 0.08 a	0.70 $\pm$ 0.07 a	0.40 $\pm$ 0.16 a	0.46 $\pm$ 0.07 a	0.31 $\pm$ 0.07 ns	0.18 $\pm$ 0.05 ns	
<b>Insecticide</b>	0.14 $\pm$ 0.07 ab	0.16 $\pm$ 0.09 ab	0.00 $\pm$ 0.00 b	0.03 $\pm$ 0.03 b	0.10 $\pm$ 0.05 b	0.20 $\pm$ 0.11 ns	0.15 $\pm$ 0.09 ns	
<b>Kaolin</b>	0.01 $\pm$ 0.01 b	0.05 $\pm$ 0.05 b	0.00 $\pm$ 0.00 b	0.03 $\pm$ 0.03 b	0.14 $\pm$ 0.04 b	0.19 $\pm$ 0.07 ns	0.18 $\pm$ 0.05 ns	
	21-Jul	25-Jul	28-Jul	2-Aug	9-Aug	16-Aug	23-Aug	
	Flowering	Harvesting	Harvesting	Harvesting	Harvesting	Harvesting	End	
<b>Control</b>	0.30 $\pm$ 0.06 ns	0.18 $\pm$ 0.05 ns	0.05 $\pm$ 0.03 ns	0.05 $\pm$ 0.03 ns	0.80 $\pm$ 0.08 ns	1.03 $\pm$ 0.11 ns	0.93 $\pm$ 0.29 ns	
<b>Insecticide</b>	0.23 $\pm$ 0.03 ns	0.30 $\pm$ 0.09 ns	0.15 $\pm$ 0.06 ns	0.05 $\pm$ 0.03 ns	0.83 $\pm$ 0.13 ns	1.03 $\pm$ 0.36 ns	1.05 $\pm$ 0.23 ns	
<b>Kaolin</b>	0.13 $\pm$ 0.03 ns	0.30 $\pm$ 0.06 ns	0.08 $\pm$ 0.05 ns	0.18 $\pm$ 0.08 ns	0.43 $\pm$ 0.12 ns	0.75 $\pm$ 0.37 ns	0.68 $\pm$ 0.23 ns	

The developmental stage abbreviations are second leaf (2L), third leaf (3L), fifth leaf (5L) and seventh to eighth leaf stage (7-8L).

Means followed by different letters are significantly different at  $P < 0.05$  according to the Tukey-Kramer test on square root transformed data (GLM; SAS Institute).

ns: non significant

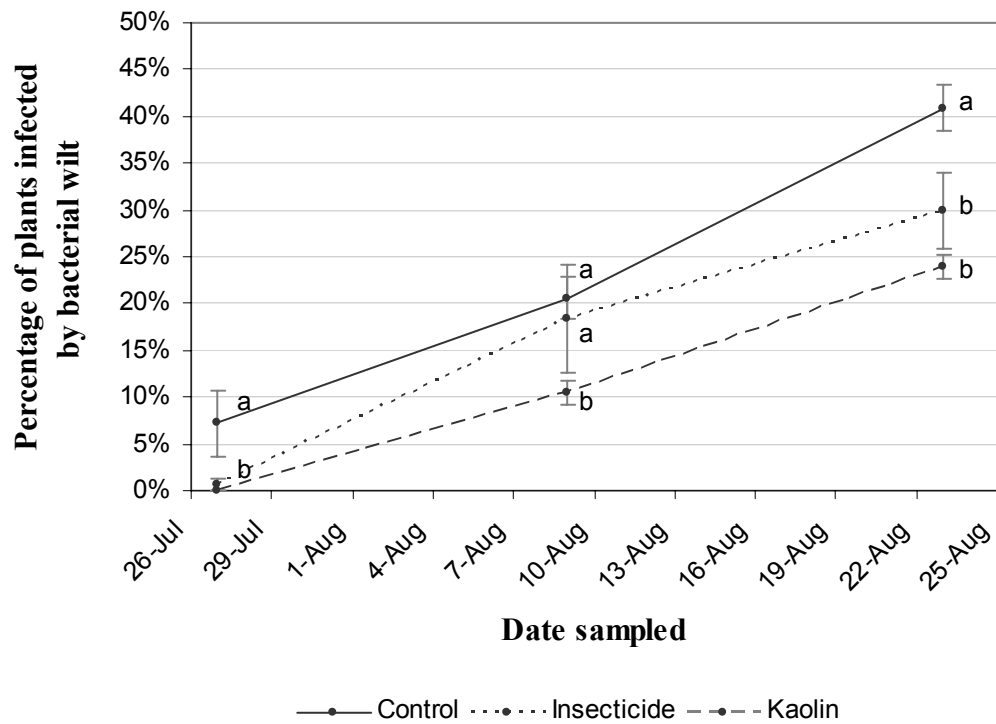


**Figure 2.2.** Effect of insecticide and kaolin on the percentage of defoliated cucumber plants (1-25% defoliation) due to striped cucumber beetles (*Acalymma vittatum*) in 2005. The developmental stage abbreviations are first leaf (1L), second leaf (2L), third leaf (3L), fifth leaf (5L) and seventh to eighth leaf stage (7-8L). For each date, values with different letters are statistically different at  $P < 0.05$  according to the binomial model (GENMOD; SAS Institute).

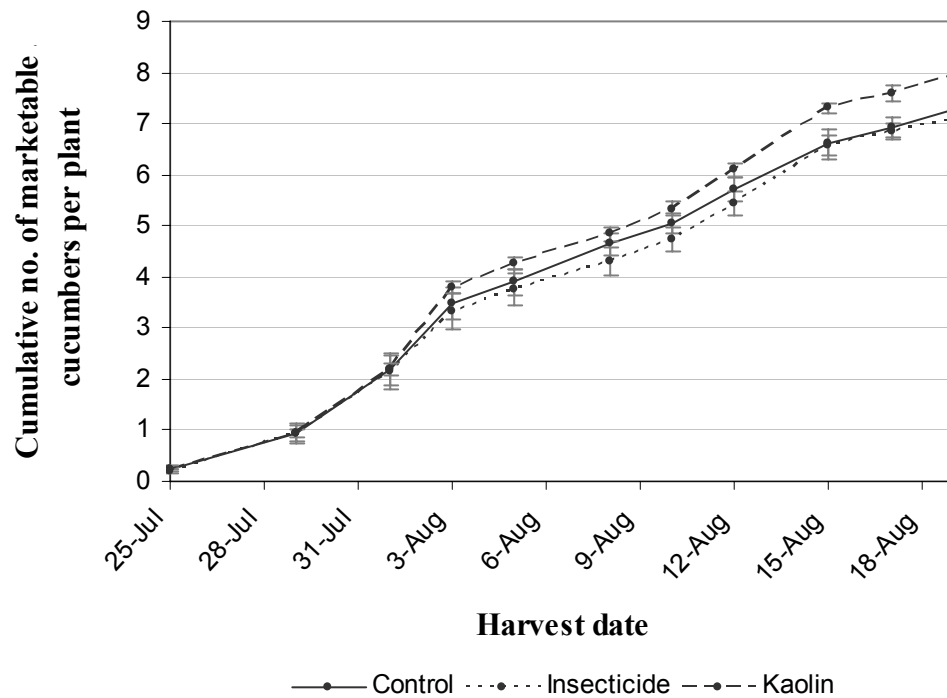
Symptoms of bacterial wilt appeared earlier in the control than in insecticide and kaolin treatments and presence of *Erwinia tracheiphila* was confirmed by the diagnostic lab. The percentage of infected plants was lower in the kaolin treatment compared with the insecticide treatment throughout the sampling period (Figure 2.3). After the first harvest (July 27), 7% of the plants were showing symptoms of bacterial wilt in control plots compared with less than 1% for the other treatments. After the sixth harvest (August 9), 21% of the plants showed symptoms of bacterial wilt in control, 18% in insecticide and significantly less 11% in the kaolin treatment. After the final harvest (August 23), control plots had 1.4 and 1.7 times as many plants infected with bacterial wilt as insecticide and kaolin-treated plots respectively. Bacterial wilt killed 7% of the plants in control treatment compared with less than 1% of the plants in the insecticide and kaolin treatments.

### ***Yields***

Yields were high in 2005 due to favorable meteorological conditions coupled with low insect and disease pressure. The cumulative yields are presented in Figure 2.4. Early production (first three harvests) was similar for all treatments and there was no difference in the total number of cucumbers per plant. The total number of marketable fruits per plant was significantly higher in the kaolin than in the two other treatments (Table 2.2). Only 1 to 2% of the cucumbers were unmarketable due to damage by SCB and there was no difference among treatments. The largest proportion (35 to 39%) of unmarketable fruit was due to yellowing. Based on observations throughout the growing season, plants grew well and had abundant foliage. This foliage may have shaded the cucumbers causing yellowing (Lin and Jolliffe 1996).



**Figure 2.3.** Effect of insecticide and kaolin on the percentage of cucumber plants infected with bacterial wilt (*Erwinia tracheiphila*) in 2005.  
For each date, points followed by different letters are statistically different at  $P < 0.05$  according to the binomial model (GENMOD; SAS Institute).



**Figure 2.4.** Effect of insecticide and kaolin on the cumulative marketable yield of cucumber (cv. Speedway) per plant grown in south western Québec in 2005.



**Table 2.2.** Effect of insecticide and kaolin on total-season number of cucumbers (cv. Speedway) per plant in 2005.

Treatment	Total	Unmarketable			Marketable		
		SCB damage	Color	Other	SCB damage	Color	Other
Control	14.3 ±0.5 ns	0.24 ±0.02 ns	5.2 ±0.3 ns	1.6 ±0.1 ns	7.3 ±0.2 b		
Insecticide	15.0 ±0.2 ns	0.32 ±0.07 ns	5.9 ±0.4 ns	1.7 ±0.1 ns	7.1 ±0.1 b		
Kaolin	15.1 ±0.1 ns	0.21 ±0.06 ns	5.3 ±0.2 ns	1.7 ±0.2 ns	7.9 ±0.1 a		

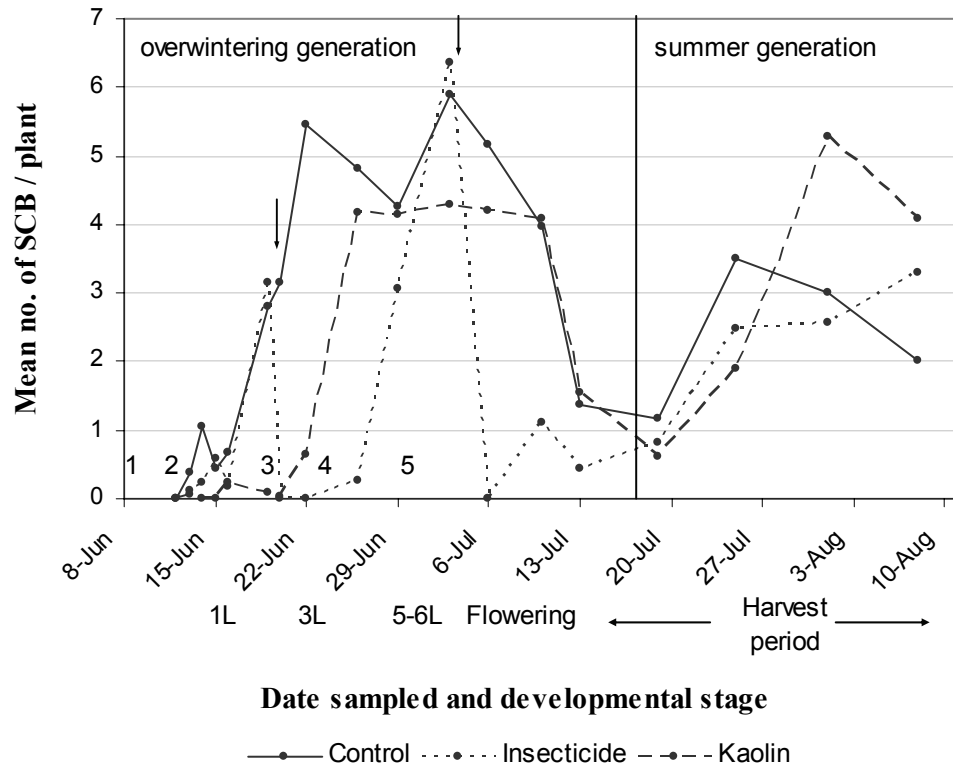
Means followed by different letters are statistically different at  $P < 0.05$  according to the Tukey-Kramer comparison test (GLM; SAS Institute).  
ns: non significant

## **2006-season**

### ***SCB populations and damage***

The 2006 season was warmer than the 29-year average but cooler than 2005 with less extreme temperatures (Appendices 1 and 3). The amount of precipitation was 1.7 times that of the 29-year average. Precipitation was unevenly distributed throughout the growing season being particular heavy at the beginning and end. During the course of the experiment, there were 9 times when more than 15 mm of rain fell and the field was flooded several times for more than twelve hours (Appendix 3).

The SCB population was high in 2006 due to both natural and introduced populations (Figure 2.5). Although each plot started with eighty introduced beetles, virtually no beetles were found in the kaolin-treated plots during the first week after the beetles were released in spite of the fact that this was the earliest cucurbit crop in the research center and such was most likely to attract newly emerging beetles. By the second week after release, beetle numbers rose dramatically in the control and insecticide plots. The overwintering generation of beetles reached a maximum of 5.9 SCB per plant in the control with on July 3, declined and then rose on July 25 to 3.5 SCB per plant on the emergence of the summer generation. Beetle numbers in the summer generation were lower than those of the overwintering generation. Beetle numbers in the insecticide treatment were initially similar to those of the control. After insecticide was sprayed on June 19, beetle numbers fell to zero and remained at that level for a week after which they rapidly increased reaching 6.4 SCB per plant at which time a second application killed the remaining insects. As the summer generation of beetles appeared, numbers slowly increased in the insecticide treatment. Beetle population in the kaolin treatment remained low until plants reach the fourth leaf stage. The kaolin coverage was well maintained during the first growth stages but at the fourth leaf stage, it became difficult to maintain adequate kaolin coverage due to the rapid rate of plant growth and development. This problem was exacerbated by the kaolin that had been applied at the fourth leaf stage being rained off on June 26, 27 and 28 (Appendix 3). After this, beetles migrated into kaolin plots and beetle density reached 4.3 SCB per plant on July 3, increasing to 5.28 SCB per plant as the summer generation emerged.



**Figure 2.5.** Effect of insecticide and kaolin on the population of adult striped cucumber beetles (SCB; *Acalymma vittatum*) on cucumber plants (cv. Speedway) grown in south western Québec in 2006. The developmental stage abbreviations are first leaf (1L), third leaf (3L) and fifth to sixth leaf stage (5-6L). The arrows indicate the dates of insecticide applications and numbers indicate the dates of kaolin applications (1 to 5).

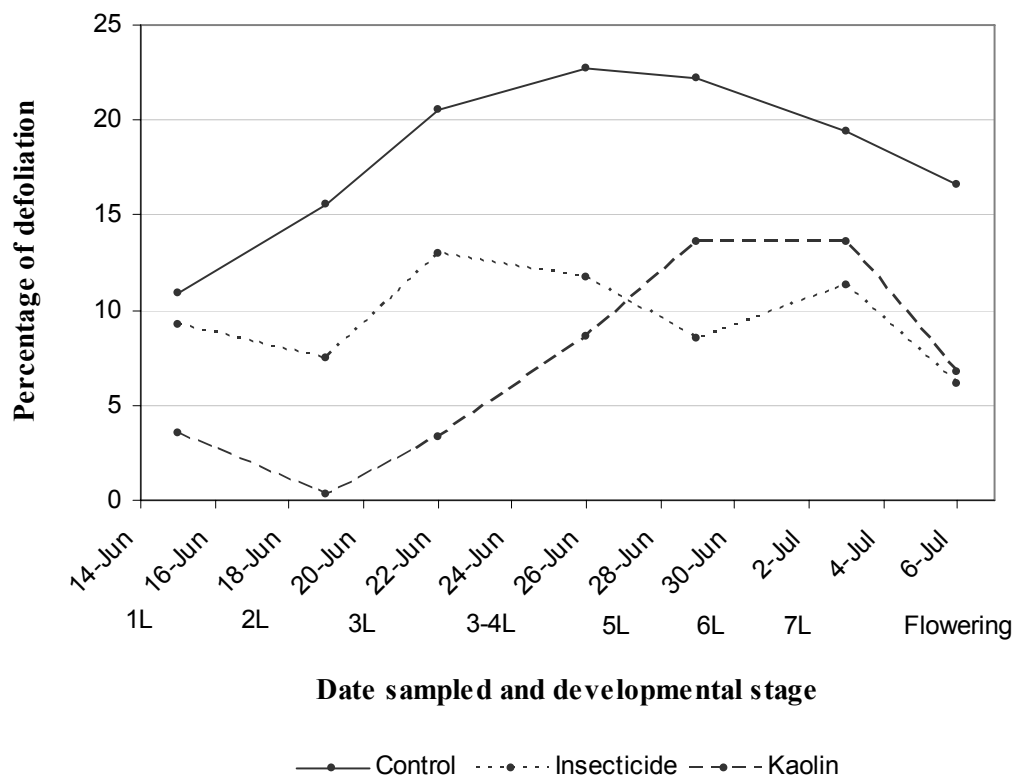
Up to the fourth leaf stage, kaolin-treated plants had significantly less SCB than the control (Table 2.3). The insecticide treatment had significantly less beetles than either the control and kaolin treatments after insecticide applications on June 26 and July 6. There was no statistical difference between seasonal means of SCB per plant of kaolin ( $2.51 \pm 0.32$ ) and insecticide ( $1.73 \pm 0.29$ ) or kaolin and control ( $3.16 \pm 0.30$ ) according to Tukey-Kramer test on square root transformed data. However, control and insecticide seasonal means were statistically different. Leaf damage in each treatment was consistent with the numbers of beetles observed. Kaolin-treated plants had less defoliation than control plants from the first leaf until flowering and lower than the insecticide-treated plants until the fifth leaf stage (Figure 2.6). There was a decrease in the percentage defoliation in the kaolin treatment between the first and third leaf stages since the total leaf area increased without new damage. In the insecticide treatment, plants had defoliation levels similar to those of the control at the start of the season (before the first insecticide application) but were significantly lower as the season progressed. Defoliation for control and insecticide treatments leveled off after the third leaf stage while the kaolin treatment showed increases in defoliation until the seventh leaf which corresponds to an increase in the number of beetles in the kaolin treatment in the same period (Figure 2.5). At the second-leaf stage, 95% of the plants had no damage in the kaolin treatment, 25% in the insecticide and only 2.5% in the control (Table 2.4). By the third leaf stage, 97.5% of the plants in the kaolin treatment had less than 11% area defoliated, 50% in the insecticide plots and 7.5% in the control plots. The proportion of plants damaged was significantly lower in the kaolin treatment (38-55%) compared with insecticide (75-95%) and control (80-100%) treatments for the most critical stages (first leaf to third leaf stages; binomial model at  $P < 0.05$ ). Less than 8% of the plants in the insecticide and kaolin treatments had greater than 25% defoliation before flowering (Table 2.4). However, from the fourth to fifth leaf stage, 30% of the control plants had greater than 25% of their leaf area defoliated. Heavy defoliation in control treatment caused a delay in the plant development as we observed a mean number of 6 leaves on kaolin and insecticide plants but 5 leaves on control plants on June 19. Also, on July 3, kaolin-treated plants had a mean of 7.7 leaves and 0.4 flowers per plant, insecticide treated plants had 8 leaves and 0.4 flowers per plant and control plants had 6.7 leaves and no flowers.

**Table 2.3.** Effect of insecticide and kaolin on the number of striped cucumber beetles (SCB; *Acalymma vittatum*) per cucumber plant in 2006 (mean  $\pm$  standard error).

Mean no. SCB / plant							
Treatment	13-Jun 1L	15-Jun 1L	19-Jun 2L	22-Jun 3L	26-Jun 4-5L	29-Jun 5-6L	3-Jul 7-8L
Control	0.35 ± 0.21 ns	0.45 ± 0.17 a	2.80 ± 0.54 a	5.45 ± 1.27 a	4.80 ± 0.39 a	4.25 ± 0.98 ns	5.90 ± 1.29 ns
Insecticide	0.13 ± 0.06 ns	0.58 ± 0.32 a	3.15 ± 1.03 a	0.00 ± 0.00 b	0.25 ± 0.12 b	3.05 ± 1.25 ns	6.35 ± 1.63 ns
Kaolin	0.05 ± 0.03 ns	0.00 ± 0.00 b	0.08 ± 0.05 b	0.65 ± 0.34 b	4.18 ± 1.56 a	4.15 ± 1.15 ns	4.30 ± 1.03 ns
	6-Jul Flowering	10-Jul Flowering	13-Jul Flowering	19-Jul Harvesting	25-Jul Harvesting	1-Aug Harvesting	8-Aug Harvesting
Control	5.15 ± 0.83 a	3.98 ± 1.36 ns	1.38 ± 0.33 a	1.18 ± 0.29 ns	3.50 ± 0.37 ns	3.00 ± 0.65 ns	2.03 ± 0.15 b
Insecticide	0.00 ± 0.00 b	1.10 ± 0.87 ns	0.43 ± 0.13 b	0.83 ± 0.17 ns	2.48 ± 0.75 ns	2.58 ± 0.39 ns	3.30 ± 0.58 ab
Kaolin	4.20 ± 0.92 a	4.08 ± 0.27 ns	1.55 ± 0.20 a	0.63 ± 0.15 ns	1.90 ± 0.63 ns	5.28 ± 1.61 ns	4.08 ± 0.50 a

The developmental stage abbreviations are first leaf (1L), second leaf (2L), third leaf (3L), fourth to fifth leaf stage (4-5L) and seventh to eighth leaf stage (7-8L). Means followed by different letters are significantly different at  $P < 0.05$  according to the Tukey-Kramer test on square root transformed data (GLM; SAS Institute).

ns: non significant



**Figure 2.6.** Effect of insecticide and kaolin on the percentage of defoliation by striped cucumber beetles (*Acalymma vittatum*) on cucumber plants (cv. Speedway) in 2006. The percentage defoliation is the weighted average of each defoliation category. The developmental stage abbreviations are first leaf (1L), second leaf (2L), third leaf (3L), third to fourth leaf stage (3-4L), fifth leaf (5L), sixth leaf (6L) and seventh leaf stage (7L).

**Table 2.4.** Effect of insecticide and kaolin on the leaf damage by the striped cucumber beetles (*Acalymma vittatum*) in 2006.

Percentages of plants in each damage category are shown by date\*.

Date	Stage	Treatment	Percentage (%) of plants in each leaf defoliation category			
			0	1-10%	11-25%	> 25%
15-Jun	1L	Control	20	40	32.5	7.5
		Insecticide	12.5	52.5	35	0
		Kaolin	62.5	30	5	2.5
19-Jun	2L	Control	2.5	40	42.5	15
		Insecticide	25	52.5	20	2.5
		Kaolin	95	5	0	0
22-Jun	3L	Control	0	7.5	75	17.5
		Insecticide	5	45	42.5	7.5
		Kaolin	45	52.5	2.5	0
26-Jun	4-5L	Control	0	15	55	30
		Insecticide	0	50	50	0
		Kaolin	12.5	57.5	30	0
29-Jun	5-6L	Control	0	10	62.5	27.5
		Insecticide	0	80	17.5	2.5
		Kaolin	10	32.5	50	7.5
3-Jul	7-8L	Control	0	10	80	10
		Insecticide	0	57.5	40	2.5
		Kaolin	12.5	25	57.5	5
6-Jul	Flowers	Control	0	32.5	57.5	10
		Insecticide	0	95	5	0
		Kaolin	0	90	10	0

The developmental stage abbreviations are first leaf (1L), second leaf (2L), third leaf (3L), fourth to fifth leaf (4-5L) and seventh to eighth leaf stage (7-8L).

\*Percentage based on a sample of 40 plants per treatment per date.

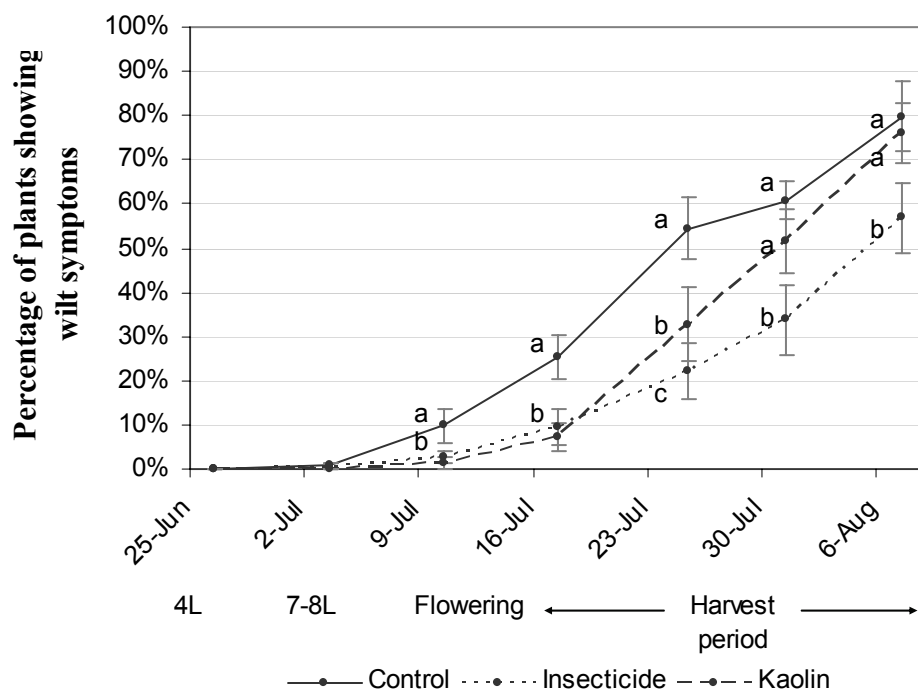
### ***Disease incidence and plant mortality***

In 2006, a disease complex was responsible for wilting symptoms and the majority of plant deaths in the field. The diagnostic lab attributed the primary cause of wilting to bacterial wilt, *Erwinia tracheiphila* (Smith), and the secondary cause to Fusarium wilt, *Fusarium oxysporum*. *F. oxysporum* is a soilborne fungus which could have entered the roots damaged by SCB larvae (Latin and Reed 1985) and is reported to flourish in warm soils (Robinson and Decker-Walters 1997). These conditions existed during the growing season as the plastic mulch enhanced the soil temperature and the repeated rains produced a wet soil which had a higher heat absorptance than dry soil (Tarara 2000; Appendix 3). Therefore, the wilt symptoms and deaths observed in the field were due to an initial infection by *E. tracheiphila* exacerbated by *Fusarium oxysporum* infection.

First symptoms of wilt appeared at the seventh to eighth leaf stage in the control plots (Figure 2.7). At the first harvest, fully 25% of the control plants were infected by wilting diseases, 10% of the insecticide and 7% of the kaolin-treated plants. After three harvests, 54% of the control plants were infected by wilting diseases, 22% of the insecticide and 33% of the kaolin-treated plants. At the last harvest, 80% of the control plants were wilted, as were 57% and 76% of the insecticide and kaolin-treated plants, respectively. Plants with a disease severity of 1 (< 20% wilt) are able to produce cucumbers however once the severity increases to levels 2 or 3 then fruit productivity is reduced. The severity level rapidly increased throughout the season (Table 2.5).

Mortality was primarily due to disease compared to insect feeding and was highest in the control plots (Figure 2.8-A). Interestingly, there was no mortality due to disease in the kaolin treatment before the sixth harvest (July 31). Up to the penultimate week, death due to disease was lower in kaolin than in the insecticide treatment. By the end of the experiment, 36% of the control, 8% of the insecticide and 16% of the kaolin-treated plants were dead. Mortality due to feeding occurred mainly during the first and second leaf stages and was significantly reduced in the kaolin (0.6 %) compared with the control (5.2%) and insecticide treatments (4.1%). Total mortality (feeding and disease) was similar for the kaolin and insecticide treatments which were lower than that of the control (Figure 2.8-B).





**Figure 2.7.** Effect of insecticide and kaolin on the percentage of cucumber plants showing wilt symptoms (*Erwinia tracheiphila* and *Fusarium oxysporum*) in 2006.

For each date, points with different letters are statistically different at  $P < 0.05$  according to the binomial model (GENMOD; SAS Institute).

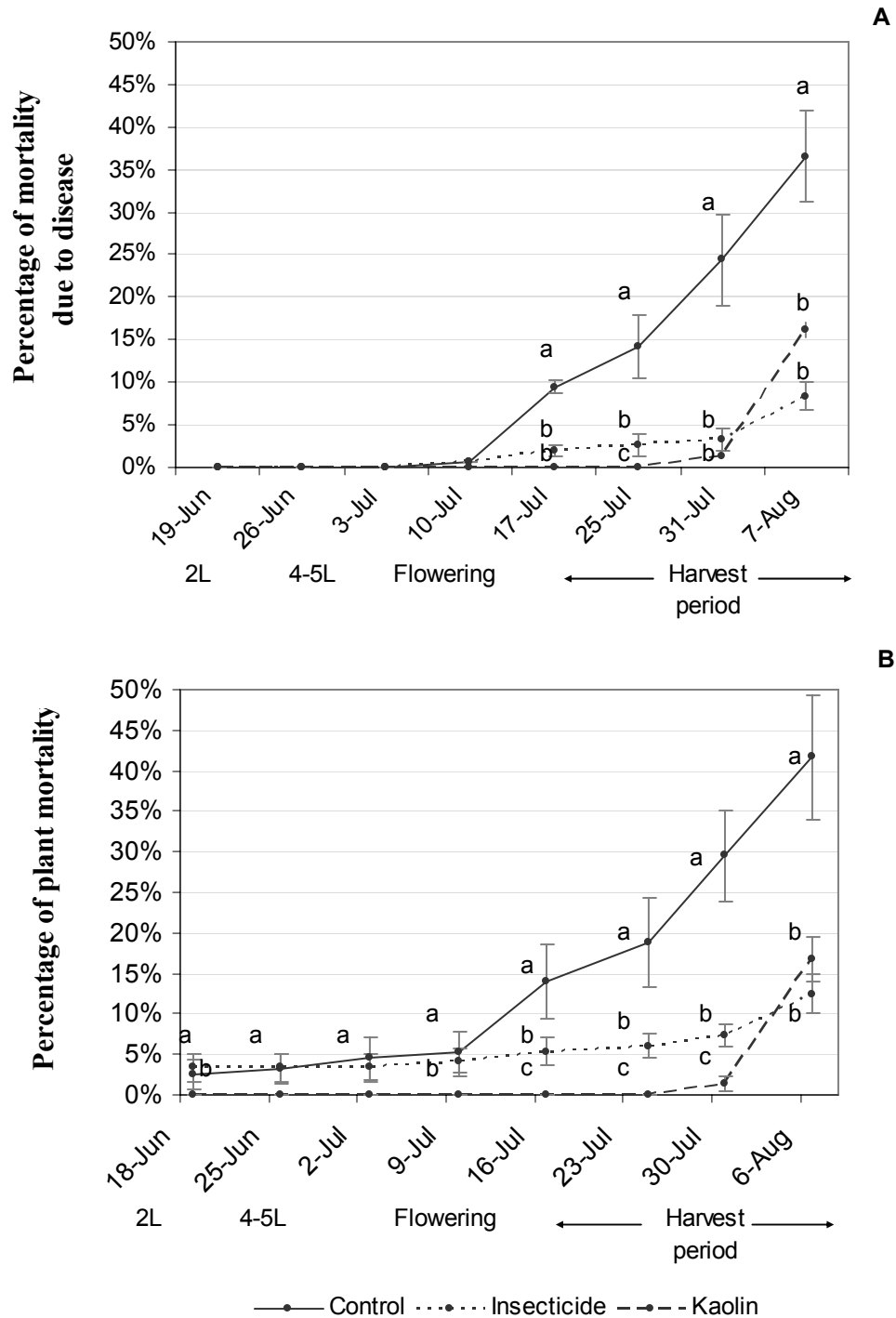
The developmental stage abbreviations are fourth (4L) and seven to eight leaf stage (7-8L).

**Table 2.5.** Effect of insecticide and kaolin on wilting symptoms severity due to *Erwinia tracheiphila* and *Fusarium oxysporum* in 2006.

Percentages of infected cucumber plants in each severity class are shown by date\*.

Date	Treatment	Percentage (%) of plants in each wilting category			
		1 > 20%	2 20-80%	3 <80%	4 Dead
<b>3-Jul</b>	Control	99.26	0	0.74	0
	Insecticide	99.38	0.63	0	0
	Kaolin	100	0	0	0
<b>10-Jul</b>	Control	90.23	4.54	4.49	0.74
	Insecticide	97.4	0.66	1.32	0.63
	Kaolin	98.75	0	1.25	0
<b>17-Jul</b>	Control	74.65	2.83	11.93	10.59
	Insecticide	90.39	4.71	2.3	2.6
	Kaolin	92.78	1.32	5.9	0
<b>25-Jul</b>	Control	45.62	9.75	26.17	18.45
	Insecticide	77.8	6.02	13.57	2.6
	Kaolin	67.26	10.99	20.5	1.25
<b>31-Jul</b>	Control	39.18	3.82	24.04	32.96
	Insecticide	66.28	1.47	25.13	7.12
	Kaolin	48.39	8.53	34.07	9.01
<b>7-Aug</b>	Control	20.21	7.02	17.84	54.94
	Insecticide	43.16	8.51	22.87	25.46
	Kaolin	23.95	3.33	37.08	35.64

\* Percentage based on the total number of plants in the middle rows of each plot.



**Figure 2.8.** Effect of insecticide and kaolin on the percentage of cucumber plant mortality due to diseases (*Erwinia tracheiphila* and *Fusarium oxysporum*) (A) and on the percentage of total cucumber mortality (B) in 2006.

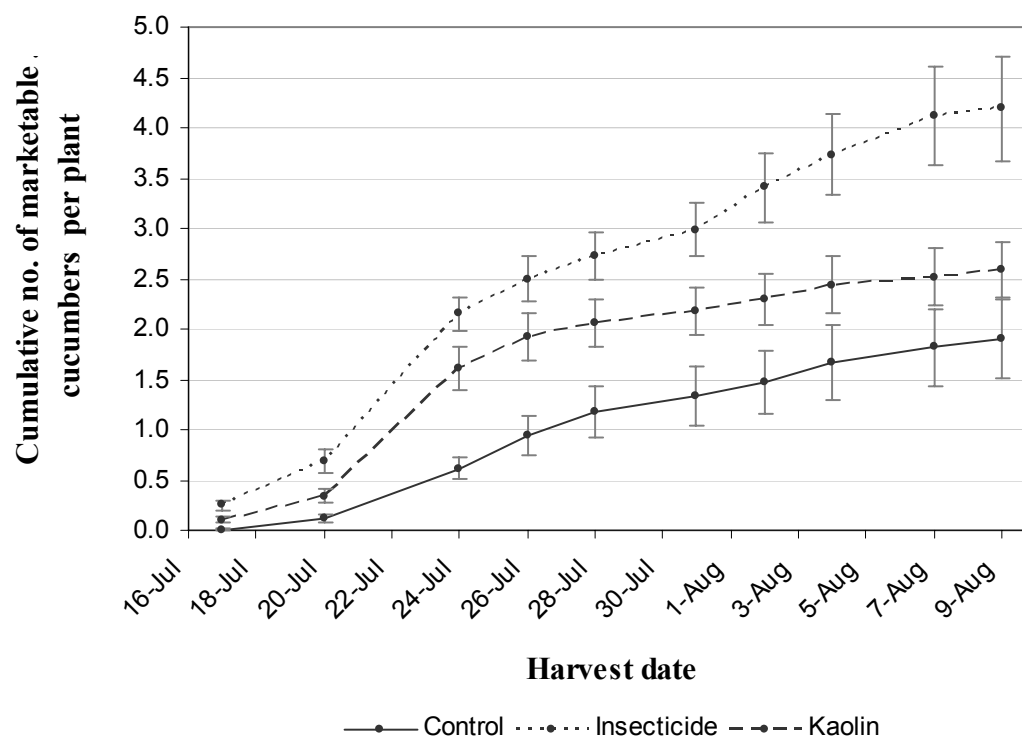
The developmental stage abbreviations are second leaf (2L) and fourth to fifth leaf stage (4-5L). For each date, points with different letters are statistically different at  $P < 0.05$  according to the binomial model (GENMOD; SAS Institute).

## ***Yields***

Yields were low in 2006, probably due to high SCB pressure and early spread of disease in conjunction with difficult climatic conditions. Early yield (first three harvests) in control plots (0.7 marketable cucumbers/ plant) was significantly lower than in kaolin (1.6) and insecticide plots (2.2) (Figure 2.9). The control treatment produced low marketable yields. The number of marketable fruits per plant in kaolin plots reached a plateau after five harvests (Figure 2.9). By this date, more than 30% of the plants were infected by wilting diseases which may explain the reduction in productivity (Figure 2.7). Up to the sixth harvest, the marketable yield was statistically similar for the kaolin and insecticide treatments. However, the total marketable yield was significantly higher in the insecticide than the other treatments (Table 2.6). The total yield in kaolin plots was not statistically different than that of the insecticide treatment (Table 2.6). The proportion of unmarketable fruits due to SCB damage was highest in the control (12%) followed by the kaolin (8%) and insecticide (6%) treatments, respectively.

## **Discussion**

The SCB seasonal mean number of beetles per plant was 8 times greater in 2006 than in 2005 in the control treatment (0.41 in 2005 vs 3.16 in 2006) since we introduced the beetles the second year. In 2006, there was a large number of overwintering beetles much greater than the subsequent summer populations. In 2006 but not in 2005, the experimental plots were the first cucurbit crop in the research center which may have attracted more of the newly emerged overwintering beetles. In addition, the overwintering beetles may have been attracted by the aggregating hormones produced by the introduced beetles (Smyth and Hoffmann 2003). In 2006, it was impossible to completely avoid migration of beetles from the overwintering generation to the kaolin plots (Figure 2.5). The rapid growth of the cucumber plants, the limited kaolin coverage coupled with the high insect populations in the control treatment may have led to beetle migration into kaolin plots. The reduction of beetle number in control plots at the end of the season may be due to a reduction in the attractiveness of those plots by August 1 because of the high



**Figure 2.9.** Effect of insecticide and kaolin on the cumulative marketable yield of cucumber (cv. Speedway) per plant grown in south western Québec in 2006.

**Table 2.6.** Effect of insecticide and kaolin on total-season number of cucumbers (cv. Speedway) per plant in 2006.

Treatment	Total	Unmarketable					Marketable				
		SCB damage					Color				
Control	2.7 ± 0.5	b	0.33 ± 0.09	ns	0.07 ± 0.03	ns	0.39 ± 0.05	b	1.9 ± 0.4	b	
Insecticide	5.6 ± 0.6	a	0.33 ± 0.11	ns	0.20 ± 0.06	ns	0.88 ± 0.10	a	4.2 ± 0.5	a	
Kaolin	3.8 ± 0.4	ab	0.28 ± 0.06	ns	0.09 ± 0.03	ns	0.79 ± 0.09	a	2.6 ± 0.3	b	

Means followed by different letters are statistically different at  $P < 0.05$  according to the Tukey-Kramer comparison test (GLM; SAS Institute).  
ns: non significant

level of plant mortality, since older and brittle leaves are not a preferred food source (Radin 1996) and other host plants were available within 300 meters.

Seedling mortality caused by insect feeding did not occur in 2005. However, in 2006, 5% and 4% of the young plants were destroyed in the control and insecticide treatments respectively. In 2005, damage caused by SCB was observed at the second leaf stage with 33% of the plants injured in the control, 23% insecticide and 11% kaolin treatments (Figure 2.2). In 2006, severe damage occurred early in the plants' development; at the first leaf stage, 80% of the control, 88% of the insecticide and 37% of the kaolin plants were damaged (Table 2.4). As a consequence of early defoliation, we observed a slower growth rate and a delay in flower production as found by Brewer et al. (1987). Burkness and Hutchison (1998) found that yields of pickling cucumbers were negatively affected when continuously defoliated (from the first-leaf stage) to 25% of their leaf area or when the plant was defoliated once to 50% of its leaf area. In 2005, we observed less than 25% defoliation throughout the season and had no yield loss. However, in 2006, the control treatment had greater than 25% defoliation between the fourth and the sixth leaf stage which could in part explain the poor yields (Table 2.4). However, neither the insecticide nor the kaolin treatment had this level of defoliation and the latter had lower yields. Therefore, yield losses in kaolin treatment may be due to bacterial and fusarium wilt presumably vectored by SCB which weaken and kill cucumbers plants causing yield losses in kaolin treatment (Figure 2.8).

In 2005, with a maximum density of 1.05 beetles per plant as many as 40% of the cucumber plants were infected by bacterial wilt in the control plots but yields were not affected. In 2006 with a maximum of 5.9 beetles per plant, 80% of the control plants showed wilt symptoms (*E. tracheiphila* exacerbated by *F. oxysporum*). A study in Alabama reported 45% of wilted cucumber vines per plants (cv. Straight 8) with a maximum of 7 beetles per plant (Yao et al. 1996). In New York state, cucumbers with maximum SCB densities of 3.6 (cv. Dasher II) and 3.3 beetles per plant (cv. Calypso) had 62 and 90% of wilted plants, respectively, on August 2 2000 and 20 days later 100% of the plants were infected (McGrath and Shishkoff 2001). Bacterial wilt sensitivity is not always correlated to the cultivar's attractiveness to beetles (McGrath 2004). Our results are similar to that study. Other studies have reported lower incidence of wilt. Brust and

Foster (1999) conducted 5 experiments in Indiana and found that no cantaloupe plant developed bacterial wilt with a density of 1 beetle per plant. When the density increased to 5 beetles per plant, less than 2% of the plants showed symptoms of bacterial wilt. The difference in the incidence of infection might be due to plant species. Bacterial wilt is more common on cucumbers and melons than squash and pumpkin (Radin 1996). Cucumber is recorded as highly susceptible but there is also variability in susceptibility among cultivars of the same species (Reiners and Petzoldt 2006). Brust and Foster (1999) observed a significant reduction in yield of cantaloupes when plants that had reached the fourth leaf stage of development were infested with 4 beetles per plant up to flowering stage. In our trials, the cucumber plants were in contact with beetles for a much longer period, from the first leaf stage until the end of harvest for control and insecticide treatments. The use of kaolin delayed the population build up to the fourth-to-fifth leaf stage but populations, thereafter, remained high until the last harvest.

Also, the severity of wilt symptoms at a specific beetle density will depend on the inoculum present i.e. the percentage of infective beetles (Yao et al. 1996). Fleischer et al. (1999) reported 7 to 11% of the soil emerging overwintering beetles were vectors of *E. tracheiphila* according to serological assays in Pennsylvania. However, the number of infected beetles increased through the growing season from 8-36% one week after transplanting to 33-78% later in the season. There was a great deal of variation in levels of infection between seasons among this 3-year study with the result that only 0-38% of the muskmelon plants developed bacterial wilt. Brust (1997) found that 1% of the muskmelon plants were infected with *E. tracheiphila* by overwintering beetles and 8-12% by the summer generation of SCB in Indiana. The results from these two studies make it difficult to conclude a general rule on the percentage of beetles infected and their ability to transmit *E. tracheiphila* due to the high variability between sites and years. We did not test the individuals of SCB to know if they were vectors of *E. tracheiphila* in our experiment. These factors may explain the large proportion of wilted plants and why yields were affected in the control and kaolin treatments. The additional problem of fusarium wilt, transmitted through roots damaged by SCB larvae feeding, accentuated the bacterial wilt symptoms and increased mortality.



Even in the insecticide treatment, 2006 yields were half those of 2005. This may be explained in part by the weather: during the crop establishment period (before fifth leaf stage) there were 6 days with over 15 mm of rain (Appendix 3). This may also be due to site differences; the 2006 field had less organic matter and was more prone to compaction accentuated by heavy rains which caused flooding. Also, the plots were surrounded by a 12-meter border of barley that was 30-40 cm high at the beginning of cucumber flowering. It is plausible that the isolation of the plots affected the pollination of the cucumbers as no hive was available within 500 meters.

However, SCB pressure was probably the main reason for the reduction in cucumber yield. SCB population built up rapidly reaching 3.15 per plant in the insecticide treatment by the third leaf stage at a point where the threshold is 0.5-1 SCB per plant. At the fifth-to-sixth leaf stage, the population had reached 3.05 SCB per plant. The threshold at this stage is from 3 to 5 beetles per plant and we decided to wait before spraying. Three days later at the next scouting, the population had reached 6.35 beetles per plant. Our experience indicates that with a high bacterial wilt probability, it is better to apply insecticide when the most conservative RAP threshold is reached (0.5 beetles per plant with less than five leaves and 3 beetles per plant over five leaves). Consequently, 57% of the plants wilted and 12% died in 2006 compared with 30% wilted and 1% dead in 2005 in insecticide treatment. Marketable yield was also reduced as 6 to 12% of the fruits were unmarketable due to SCB damage in 2006 compared with 1-2% in 2005.

In conclusion, kaolin can be used as an additional tool to protect cucurbit seedlings from SCB attack. When applied during the critical developmental stages, kaolin reduced SCB counts and plant damage. This, in turn, delayed bacterial wilt infection and increased the time allowed for plant development which insured good flower production and fruit formation. With low SCB populations, it is possible to obtain satisfactory results with this control method. However, with high SCB populations, it would be necessary to combine kaolin use with another control method to protect plants when the foliage is too abundant to allow for good kaolin coverage. Kaolin by itself would not be recommendable on a site with a history of heavy bacterial wilt problems. It is possible to use kaolin as a preventative measure of protection for seedlings or transplants and combine its use with other control methods depending on the levels of beetle infestation.

## CHAPTER THREE

### Evaluation of the impact of kaolin film on leaf gas exchange and growth of cucumber plants

#### Introduction

Kaolin (kaolinite) is a clay of the type aluminosilicate with non-abrasive particles which disperse easily in water (Glenn and Puterka 2005). A formulation of kaolin was developed for use in agriculture to protect crops from pests and diseases and for protection of fruit against sunburn. The clay forms a white and porous film which disrupted the insects' host finding abilities by changing visual, tactile, gustative or olfactory cues of the host plant (Glenn et al. 1999; Puterka et al. 2000; Puterka et al. 2005). The primary mechanism of action found was the repellence of insects from treated foliage (Glenn and Puterka 2005). Kaolin has been used successfully to control pear psylla, *Cacopsylla pyricola* (Glenn et al. 1999; Puterka et al. 2000; Puterka et al. 2005), apple maggot fly, *Rhagoletis pomonella* (Puterka et al. 2000; Garcia et al. 2004), and codling moth, *Cydia pomonella* (Puterka et al. 2000; Unruh et al. 2000; Friedrich et al. 2003), in apple and pear orchards, glassy winged sharpshooter, *Homalodisca coagulata*, in grapes (Puterka et al. 2003), boll weevil *Anthonomus grandis* in cotton (Showler 2002a) and others.

Kaolin film has to cover the entire plant surface to efficiently prevent insect damage. Its effects on plant growth and development have been variable. Kaolin has been shown to have beneficial effects on the growth of plants, especially under warm and dry conditions. Those effects are attributed to the capacity of kaolin to reduce stress of plants exposed to excessive temperature by improving net photosynthesis (Erez and Glenn 2004). Kaolin reduced leaf temperature and enhanced carbon assimilation, stomatal conductance and transpiration of apple and citrus leaves under heat stress (Glenn et al. 2001a; Jifon and Syvertsen 2003). On pecan trees (*Carya illinoensis* 'Pawnee'), kaolin reduced the leaf temperature but had no effect on leaf carbon assimilation, stomatal conductance, yield or the quality of the nuts (Lombardini et al. 2005). Russo and Diaz-Pérez (2005) found no difference in carbon assimilation, stomatal conductance,

transpiration, leaf temperature and yields when kaolin was applied to peppers (*Capsicum annuum* L.). On the other hand, kaolin was reported to delay tomato fruit development (Makus 2000) and reduce apple fruit size (Schupp et al. 2002). Although kaolin has been used to control insects in the Cucurbitaceae family there are few reports on its affect on the plant itself. Therefore experiments were designed to determine the effect of differing rates of kaolin as single and multiple applications on the leaf gas exchange and growth and development of cucumber (*Cucumis sativus* L.).

## **Material and Methods**

The experiments were conducted in February and March 2006 in a greenhouse on the Macdonald Campus of McGill University in Sainte-Anne-de-Bellevue, Québec (lat. 45° 26'N, long. 73° 56'W). The temperature of the greenhouse was maintained at 25/22 ± 2°C (day/night). A day length of 13 hours was used in the experiment; high pressure sodium lamps (P.L. Light Systems, Beamsville, Ontario) were used providing around 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of light at the canopy level.

### ***Experiment 1: The effect of different rates of kaolin applied at the second-leaf stage***

Cucumbers cv. Speedway (Semences B.C., Laval, Québec) were sown into 25 x 25 x 25 mm peat pots filled with Pro-Mix BX (Premier Horticulture, Rivière-du-Loup, Québec). The trays were covered with plastic until the seedling emerged. Eight days after emergence, forty-eight uniform cotyledon-stage plants were transplanted into 400 cc pots (Nursery Supplies Inc., Chambersburg, PA, USA) filled with Pro-Mix BX. Plants were placed on a bench, watered as required and fertilized weekly with 200 ml of soluble fertilizer (183 ppm N - 48 ppm P - 224 ppm K; Plant Products Company Ltd., Brampton, Ontario). The experiment was designed as a randomized complete block design with 4 blocks and 2 replications of each of the 6 treatments per block. Different rates of kaolin (Surround® WP; Engelhard Corp., Iselin, NJ, USA) were applied at the second-leaf stage (February 12, 2006): control, 12 g/L, 25g/L, 38 g/L, 50 g/L, 62 g/L. Plants were sprayed until run-off with a carbon dioxide (CO<sub>2</sub>) research gun sprayer at 30 PSI using a fine droplets XR TeeJet 8001 nozzle (Spraying Systems Co., Wheaton, IL, USA). Leaf gas exchange measurements such as leaf net carbon assimilation rate as measure of

photosynthesis, stomatal conductance, transpiration rate, intercellular CO<sub>2</sub> concentration, vapor pressure deficit and leaf temperature were measured on the second-leaf daily for the first three days, then five and ten days after kaolin application with a portable photosynthesis system (LI-6400; LI-COR Biosciences Inc., Lincoln, NE, USA) equipped with a standard leaf chamber (encloses 6 cm<sup>2</sup> of leaf area) and CO<sub>2</sub> injection system (model 6400-01; LI-COR Biosciences Inc., Lincoln, NE, USA) adjusted to a constant CO<sub>2</sub> concentration of 400 µmol CO<sub>2</sub> mol air<sup>-1</sup>. The light intensity for all measurements was 800 µmol m<sup>-2</sup> s<sup>-1</sup> provided by a red-blue light source (model 6400-02; LI-COR Biosciences Inc., Lincoln, NE, USA). After enclosing the leaf in the chamber, 2 to 3 minutes were allowed for the photosynthetic rate to stabilize. All measurements of leaf gas exchange were performed between 10:00 and 13:00 h. Seventeen days after treatment, plants were cut below the cotyledonary node and fresh weight, plant length and leaf area were recorded. Leaf area measurements were taken on each leaf with a leaf area meter with conveyer belt unit (Delta-T Devices Ltd., Burwell, Cambridge, UK). Plant material was dried at 50°C for 3 days and dry weight measured.

***Experiment 2: Single and multiple kaolin applications at different plant growth stages.***

Cucumbers cv. Speedway (Semences B.C., Laval, Québec) were sown in 50 square plug trays 54.6 x 27.9 x 6.1 cm (ITML Horticultural Products Inc., Brantford, Ontario) in Pro-Mix BX (Premier Horticulture, Rivière-du-Loup, Québec) topped with a plastic cover until emergence. Nineteen days after seeding, seventy uniform cotyledon-to-first-leaf stage plants were planted into 1000 cc pots (Nursery Supplies Inc., Chambersburg, PA, USA) filled with Pro-Mix BX. The experimental design was a randomized complete block design (RCBD) with 10 replications. Each plant within a block was randomly assigned one of the seven treatments. Plants were watered as required and fertilized weekly with 300 ml of soluble fertilizer (183 ppm N- 48 ppm P- 224 ppm K; Plant Products Company Ltd. Brampton, Ontario). Kaolin (Surround<sup>®</sup> WP; Engelhard Corp., Iselin, NJ, USA) was sprayed at the rate of 50 g/L with the same sprayer and methodology used in the first experiment. This rate was chosen as it is the maximum rate recommended for striped cucumber beetle control (PMRA 2006). Plants were sprayed until run off once, twice or three times at different phenological stages. Treatments are described in Table 3.1.

**Table 3.1.** Growth stage of cucumber plants and date of kaolin application in experiment 2

<b>Treatments</b>		<b>Date of applications</b>		
	<b>Stage of application</b>	<b>First application</b>	<b>Second application</b>	<b>Third application</b>
1	Control			
2	2L	20-Mar		
3	3L	26-Mar		
4	4L	29-Mar		
5	2L + 3L	20-Mar	26-Mar	
6	3L + 4L	26-Mar	29-Mar	
7	2L + 3L + 4L	20-Mar	26-Mar	29-Mar

The developmental stage abbreviations are second leaf (2L), third leaf (3L) and fourth leaf stage (4L).

Leaf net carbon assimilation rate, stomatal conductance transpiration rate, intercellular CO<sub>2</sub> concentration, vapor pressure deficit and leaf temperature were measured with a portable photosynthesis system (LI-6400) as outlined in experiment 1. Readings were taken on the second and the third-leaf 24 hours after kaolin applications. Due to technical problems with the portable photosynthesis system, data on the third-leaf after the 4L-stage application was taken only after 48 hours. Twelve days after the first treatment, plants were cut under the cotyledonary node and plant material was collected. Fresh weight, plant length, leaf area and dry mass were recorded following the same methodology used in experiment 1. Length and width of the leaves was measured before each kaolin application and at the end of the experiment. A regression equation was obtained by plotting length\*width product and the leaf area measured of the control plant leaves at the end of the experiment. These equations were used to estimate leaf areas and calculate leaf growth rates.

***Statistical Analyses.*** SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses with the GLM (General Linear Model) procedure. Data were tested for homogeneity of variance and normality and then subjected to an analysis of variance. Lsmeans were used when there was missing data. Multiple comparisons were conducted with Tukey-Kramer test at the probability level of 5%.

## **Results**

### ***Experiment 1***

Gas exchange measurements on kaolin-treated leaves showed a reduction in carbon assimilation, stomatal conductance and transpiration, during the three days after kaolin application (Table 3.2). One day after spraying, the 62 g/L application significantly reduced the carbon assimilation rate compared to the control and the 12 g/L kaolin treatment. However, no statistical differences were found for stomatal conductance, intercellular CO<sub>2</sub> concentration, transpiration, vapor pressure deficit and leaf temperature. Two days after spraying, a significant reduction in carbon assimilation rate between the control leaf and the 25g/L kaolin-treated leaf was observed. The carbon assimilation was not statistically different between the control, and the 38, 50 and 62 g/L kaolin-treated plants. Stomatal conductance, intercellular CO<sub>2</sub> concentration, transpiration, vapor

pressure deficit and leaf temperature did not differ among treatments. The third day after spraying, there was no statistical difference among treatments for carbon assimilation, intercellular CO<sub>2</sub> concentration, vapor pressure deficit and leaf temperature. However, control plants had higher rates of stomatal conduction than all of the kaolin-treated plants but the rates were only significantly higher for the 12, 25 and 62 g/L kaolin treatments. The control had significantly higher rates of transpiration than the 12g/L kaolin-treated plants. No significant difference was found for any of the parameters measured five and ten days after the kaolin spray.

The gas exchange measurements did not show linear responses to increasing kaolin application rates. This may be due to the difficulty in obtaining a uniform coverage when spraying kaolin even with a strict application protocol. The exact particle density on the leaf surface was not directly assessed during this experiment. From our observations, the possible difference in the kaolin coverage between the treatments may explain the lack of relationship between application rate and leaf gas exchange. The kaolin treatments were pooled since there were few statistical differences among them with the exception of carbon assimilation one day after spraying. Figure 3.1 shows the effect leaf gas exchange parameters and leaf temperature for kaolin versus untreated leaves.

For the first two days after the application, carbon assimilation was significantly reduced but stomatal conductance and transpiration were reduced only slightly. Also, intercellular CO<sub>2</sub> concentration was increased in the kaolin-treated leaves. The third day after treatment, stomatal conductance and transpiration were significantly lower for the kaolin-treated leaves. Intercellular CO<sub>2</sub> concentration was lower and vapor pressure deficit was significantly higher in the kaolin-treated leaves but carbon assimilation was not affected. The fifth and tenth days after kaolin application, the effect of kaolin was negligible and did not differ significantly from the controls.

A single application of kaolin at the second-leaf stage did slightly but not significantly reduce fresh and dry weights and leaf area but had variable effects on plant length (Table 3.3).

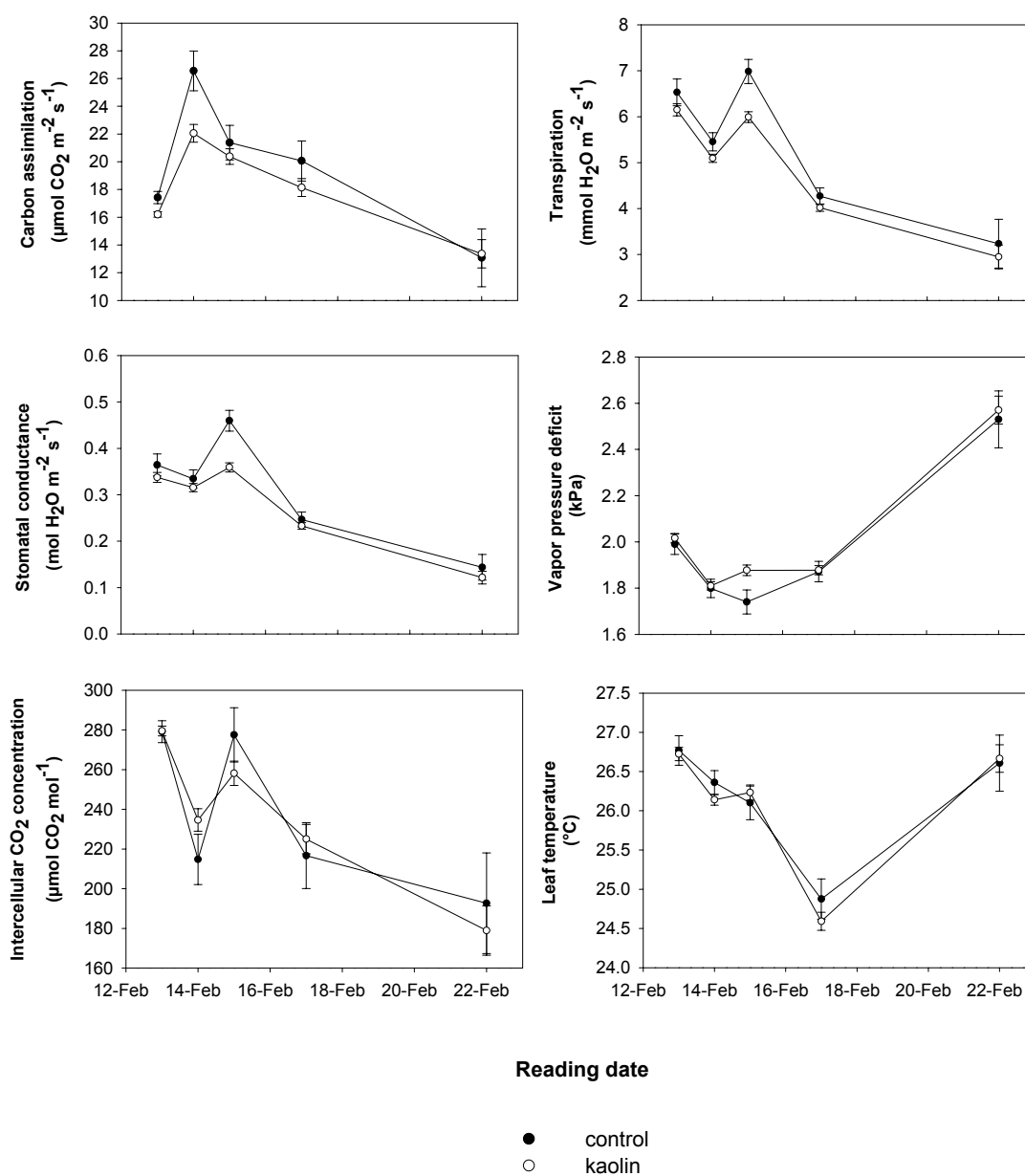
**Table 3.2.** Effect of a single kaolin application and time after application on leaf gas measurements and leaf temperature of the second leaf of cucumber cv. Speedway.

Treatment	Day 1		Day 2		Day 3		Day 5		Day 10	
	13-Feb		14-Feb		15-Feb		17-Feb		22-Feb	
Carbon assimilation (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )										
control	17.43	a	26.55	a	21.38	ns	20.06	ns	13.08	ns
12 g/L	17.44	a	22.30	ab	19.68	ns	18.90	ns	13.47	ns
25 g/L	16.18	ab	19.48	b	21.24	ns	16.96	ns	13.73	ns
38 g/L	15.79	ab	22.43	ab	20.23	ns	18.14	ns	13.62	ns
50 g/L	16.05	ab	22.73	ab	19.84	ns	19.34	ns	12.65	ns
62 g/L	15.56	b	23.33	ab	20.90	ns	17.38	ns	13.50	ns
Stomatal conductance (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )										
control	0.364	ns	0.334	ns	0.460	a	0.247	ns	0.144	ns
12 g/L	0.339	ns	0.309	ns	0.345	b	0.229	ns	0.130	ns
25 g/L	0.354	ns	0.323	ns	0.349	b	0.242	ns	0.126	ns
38 g/L	0.325	ns	0.317	ns	0.377	ab	0.236	ns	0.132	ns
50 g/L	0.366	ns	0.333	ns	0.384	ab	0.232	ns	0.117	ns
62 g/L	0.304	ns	0.295	ns	0.340	b	0.226	ns	0.110	ns
Intercellular CO <sub>2</sub> concentration (μmol CO <sub>2</sub> mol <sup>-1</sup> )										
control	279.13	ns	214.75	ns	277.50	ns	216.63	ns	192.67	ns
12 g/L	272.38	ns	234.25	ns	246.94	ns	208.25	ns	202.65	ns
25 g/L	284.50	ns	252.63	ns	250.25	ns	247.63	ns	183.00	ns
38 g/L	279.63	ns	226.78	ns	268.63	ns	220.88	ns	205.65	ns
50 g/L	284.50	ns	240.13	ns	270.00	ns	215.63	ns	171.67	ns
62 g/L	276.25	ns	219.25	ns	254.75	ns	232.38	ns	153.27	ns
Transpiration (mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )										
control	6.53	ns	5.45	ns	6.98	a	4.27	ns	3.23	ns
12 g/L	6.17	ns	5.03	ns	5.72	b	3.95	ns	3.18	ns
25 g/L	6.27	ns	5.19	ns	5.98	ab	4.07	ns	3.04	ns
38 g/L	5.95	ns	5.10	ns	6.08	ab	4.03	ns	3.20	ns
50 g/L	6.55	ns	5.29	ns	6.26	ab	4.06	ns	2.76	ns
62 g/L	5.81	ns	4.86	ns	5.92	ab	3.98	ns	2.76	ns
Vapor pressure deficit (kPa)										
control	1.99	ns	1.80	ns	1.74	ns	1.87	ns	2.53	ns
12 g/L	2.01	ns	1.82	ns	1.91	ns	1.90	ns	2.49	ns
25 g/L	1.97	ns	1.83	ns	1.93	ns	1.81	ns	2.51	ns
38 g/L	2.03	ns	1.81	ns	1.80	ns	1.88	ns	2.46	ns
50 g/L	2.00	ns	1.77	ns	1.83	ns	1.90	ns	2.65	ns
62 g/L	2.08	ns	1.82	ns	1.92	ns	1.91	ns	2.66	ns
Leaf temperature (°C)										
control	26.77	ns	26.36	ns	26.10	ns	24.88	ns	26.61	ns
12 g/L	26.76	ns	26.08	ns	26.22	ns	24.71	ns	26.34	ns
25 g/L	26.55	ns	26.33	ns	26.56	ns	24.07	ns	26.45	ns
38 g/L	26.72	ns	26.14	ns	25.87	ns	24.53	ns	26.20	ns
50 g/L	26.77	ns	26.08	ns	26.04	ns	24.81	ns	26.96	ns
62 g/L	26.82	ns	26.08	ns	26.47	ns	24.84	ns	27.03	ns

Lsmeans are an average of 8 plants except for day 10 which are an average of 6 plants.

Different letters show significant treatment difference based on Tukey-Kramer test at  $P < 0.05$ .





**Figure 3.1.** Effect of a single kaolin application at the second leaf stage (February 12) on the second leaf carbon assimilation, stomatal conductance, intercellular CO<sub>2</sub> concentration, transpiration, vapor pressure deficit and leaf temperature of cucumber cv. Speedway. All kaolin treatments (different concentrations) pooled together. For control, lsmeans ( $\pm$  SE) are based on 8 plants except for February 22 when they are based on 6 plants. For kaolin, lsmeans ( $\pm$  SE) are based on 40 plants except for February 22 when they are based on 30 plants.

**Table 3.3.** Effect of different rates of kaolin on weight, length and leaf area of cucumber cv. Speedway 17 days after application.

<b>Treatment</b>	<b>Fresh weight (g)</b>	<b>Plant length (cm)</b>	<b>Leaf area (cm<sup>2</sup>)</b>	<b>Dry mass (g)</b>
Control	41.9 ± 2.9	61.8 ± 3.7	1051 ± 54.6	5.99 ± 0.33
12g/L	36.9 ± 2.0	57.1 ± 3.0	883.8 ± 46.6	5.37 ± 0.30
25 g/L	37.5 ± 2.4	56.6 ± 5.6	926.0 ± 49.0	5.63 ± 0.40
38 g/L	39.0 ± 3.8	63.1 ± 5.0	841.0 ± 72.8	5.74 ± 0.47
50 g/L	35.5 ± 2.5	55.3 ± 3.1	853.5 ± 63.3	5.35 ± 0.32
62 g/L	35.6 ± 3.4	57.6 ± 4.5	861.8 ± 135.1	5.27 ± 0.45
<i>Probability</i>	<i>0.6426</i>	<i>0.5141</i>	<i>0.0699</i>	<i>0.6257</i>

Each mean (±SE) is an average from 8 plants except for leaf area which is an average from 4 plants.  
Treatments comparisons are based on Tukey-Kramer test.

## ***Experiment 2***

Plants that had been sprayed with kaolin at the second leaf stage had a significantly lower carbon assimilation rate than the untreated control leaf (Table 3.4). Stomatal conductance, intercellular CO<sub>2</sub> concentration, transpiration, vapor pressure deficit and leaf temperature did not differ from those of the control leaves. However, intercellular CO<sub>2</sub> concentration was slightly higher in the kaolin-treated than control leaves. This result is consistent with that of experiment 1 where carbon assimilation was reduced 24 hours after the leaf was sprayed. After the third leaf stage spray, the second leaf, which now had two layers of kaolin, had a significantly lower rate of carbon assimilation and a significantly higher intercellular CO<sub>2</sub> concentration 24 hours after the second application. However, plants that had been sprayed seven days previous (2L) had leaf gas exchange measurements equivalent to that of the control. Spraying the plant at the fourth-leaf stage, slightly but not significantly reduced carbon assimilation, stomatal conductance and transpiration but increased intercellular CO<sub>2</sub> concentration of the second leaf compared with the controls.

Twenty four hours after being sprayed, the third leaf (Table 3.5) had values for carbon assimilation, stomatal conductance, transpiration, vapor pressure deficit and leaf temperature statistically similar to those of the control. Forty-eight hours after the fourth leaf stage spray, the third leaf which had a second layer of kaolin did not show any difference from the control in terms of gas exchange.

In experiment 2, when plants that had been sprayed at the second and third leaf stage (2L + 3L) are compared with plants sprayed at 2L + 3L +4L, they are equivalent in terms of carbon assimilation (Table 3.4). This is interesting in the fact that the former has one less layer of kaolin and had a longer period to recover from spraying. This suggests that two layers of kaolin have a shading effect that may affect carbon assimilation for a longer period.

There was no significant difference in terms of fresh weight, plant length, leaf area and dry weight among the kaolin treatments and the control plants at the end of the experiment (Table 3.6). We observed that the second and third leaves grew faster and reached a larger final area than the first leaf (Table 3.7). This difference in growth rate of cucumber leaves may help explain the different responses to applications of kaolin.

**Table 3.4.** Effect of single and multiple kaolin applications on gas exchange measurements and leaf temperature of the second leaf of cucumber cv. Speedway.

		21-Mar		27-Mar		30-Mar	
Treatment		1 day after 2L-stage spray		1 day after 3L-stage spray		1 day after 4-L stage spray	
<b>Carbon assimilation</b> ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )							
1	Control	15.05	a	19.67	a	21.30	
2	2L	13.85	b	20.01	a	18.76	
5	2L+3L			16.47	b	15.76	
7	2L+3L+4L					16.40	
	<i>Pr</i>	0.0122		0.0106		0.0918	
<b>Stomatal conductance</b> ( $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ )							
1	Control	0.245		0.246		0.128	
2	2L	0.250		0.234		0.149	
5	2L+3L			0.225		0.135	
7	2L+3L+4L					0.114	
	<i>Pr</i>	0.8040		0.6333		0.4543	
<b>Intercellular CO<sub>2</sub> concentration</b> ( $\mu\text{mol CO}_2 \text{ mol}^{-1}$ )							
1	Control	263.5		223.2	ab	140.0	
2	2L	278.0		215.2	b	151.3	
5	2L+3L			238.8	a	174.0	
7	2L+3L+4L					155.7	
	<i>Pr</i>	0.0856		0.0173		0.7948	
<b>Transpiration</b> ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ )							
1	Control	3.148		4.487		1.517	
2	2L	3.116		4.361		1.602	
5	2L+3L			4.143		1.528	
7	2L+3L+4L					1.303	
	<i>Pr</i>	0.8730		0.4919		0.4741	
<b>Vapor pressure deficit</b> (kPa)							
1	Control	1.404		1.992		1.208	
2	2L	1.335		2.014		1.091	
5	2L+3L			2.002		1.173	
7	2L+3L+4L					1.169	
	<i>Pr</i>	0.0739		0.8932		0.2252	
<b>Leaf temperature</b> ( $^{\circ}\text{C}$ )							
1	Control	26.58		26.82		27.29	
2	2L	26.21		26.85		26.63	
5	2L+3L			26.60		27.12	
7	2L+3L+4L					26.84	
	<i>Pr</i>	0.0805		0.3137		0.0878	

Values followed by different letters are statistically different at  $P < 0.05$  according to Tukey-Kramer multiple comparisons test.

Values presented are LSMEANS of 10 leaves on March 21, 6 leaves on March 27 and 5 leaves on March 30.

The developmental stage abbreviations are second leaf (2L), third leaf (3L) and fourth leaf stage (4L).

**Table 3.5.** Effect of single and multiple kaolin applications on gas exchange measurements and leaf temperature of the third leaf of cucumber cv. Speedway.

Treatment	27-Mar	31-Mar
	1 day after 3L-stage spray	2 days after 4L-stage spray
<b>Carbon assimilation (<math>\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}</math>)</b>		
1 Control	19.53	17.72
3 3L	17.50	16.23
6 3L+4L		14.60
<i>Pr</i>	0.0807	0.1082
<b>Stomatal conductance (<math>\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}</math>)</b>		
1 Control	0.265	0.218
3 3L	0.296	0.195
6 3L+4L		0.184
<i>Pr</i>	0.4350	0.4481
<b>Intercellular CO<sub>2</sub> concentration (<math>\mu\text{mol CO}_2 \text{ mol}^{-1}</math>)</b>		
1 Control	233.2	231.2
3 3L	258.0	228.3
6 3L+4L		236.6
<i>Pr</i>	0.0688	0.6738
<b>Transpiration (<math>\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}</math>)</b>		
1 Control	4.432	2.864
3 3L	4.490	2.525
6 3L+4L		2.526
<i>Pr</i>	0.8329	0.2695
<b>Vapor pressure deficit (kPa)</b>		
1 Control	1.841	1.398
3 3L	1.747	1.390
6 3L+4L		1.448
<i>Pr</i>	0.3478	0.7088
<b>Leaf temperature (°C)</b>		
1 Control	27.32	28.24
3 3L	26.94	28.01
6 3L+4L		28.31
<i>Pr</i>	0.4329	0.5582

Values presented are LSMEANS of 9 leaves on March 27 and 5 leaves on March 31. The developmental stage abbreviations are third leaf (3L) and fourth leaf stage (4L).

**Table 3.6.** Effect of single and multiple kaolin applications on weight, length and leaf area of cucumber cv. Speedway 12 days after the first application.

Treatment		Plant length (cm)	Fresh weight (g)	Leaf area (cm <sup>2</sup> )	Dry mass (g)
1	Control	24.8 ± 2.8	41.3 ± 3.2	944 ± 69	5.65 ± 0.41
2	2L	26.4 ± 2.8	44.1 ± 3.2	1013 ± 64	5.78 ± 0.41
3	3L	22.6 ± 2.8	36.4 ± 3.2	818 ± 64	4.58 ± 0.41
4	4L	21.7 ± 3.0	42.1 ± 3.5	1004 ± 64	5.56 ± 0.44
5	2L + 3L	21.0 ± 2.8	35.8 ± 3.2	851 ± 64	4.48 ± 0.41
6	3L + 4L	23.6 ± 2.8	43.0 ± 3.2	982 ± 64	5.33 ± 0.41
7	2L + 3L + 4L	24.1 ± 2.8	39.5 ± 3.2	922 ± 64	4.86 ± 0.41
<i>Probability</i>		<i>0.8779</i>	<i>0.5227</i>	<i>0.2509</i>	<i>0.1549</i>

LSMEANS (±SE) are based on an average of 8 plants except some missing data.

Treatments comparisons are based on Tukey-Kramer test.

The developmental stage abbreviations are second leaf (2L), third leaf (3L) and fourth leaf stage (4L).

**Table 3.7.** Leaf area of the untreated control plants estimated at the application date and measured at the end of the experiment and growth rate of the different leaves of cucumber cv. Speedway.

	First leaf		Second leaf		Third leaf		Fourth leaf	
	leaf area (cm <sup>2</sup> )	Date	leaf area (cm <sup>2</sup> )	Date	leaf area (cm <sup>2</sup> )	Date	leaf area (cm <sup>2</sup> )	Date
before spray*	36.24	20-Mar	53.68	20-Mar	192.42	26-Mar	199.29	29-Mar
End of experiment	41.5	31-Mar	191.1	31-Mar	302.3	31-Mar	259	31-Mar
growth	5.26	11 days	137.42	11 days	109.88	5 days	59.71	2 days
growth rate	0.48	cm <sup>2</sup> /day	12.49	cm <sup>2</sup> /day	21.97	cm <sup>2</sup> /day	29.86	cm <sup>2</sup> /day

\* leaf area before the kaolin application is estimated by regression equations (length\*width x leaf area measured) for each leaf of the control plants

## Discussion

We observed a significant reduction in carbon assimilation in the second leaf 24 hours after kaolin application in both experiments. Once inside the leaf, the CO<sub>2</sub> remains in the intercellular spaces because it is not used for the photosynthetic process. The gas exchange parameters were measured below light saturation (800  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) with an air temperature of 24 to 30°C, conditions that are optimal for photosynthesis (Tesar 1984). Therefore, kaolin may create shade and reduce the light reaching the chloroplasts, exposing the leaf to suboptimal conditions for photosynthesis. Glenn et al. (1999) evaluated that kaolin can reflect 10% of the photosynthetically active radiations (PAR) reducing light available to chloroplasts. At lower light intensity, leaf carbon assimilation can be reduced due to less light reaching the leaf surface (Glenn et al. 1999) because CO<sub>2</sub> assimilation is limited by low light level rather than excessive heat. Similarly, Wünsche et al. (2004) noted a decrease in carbon assimilation but no effect on transpiration and stomatal conductance on kaolin-treated apple leaves and they attributed this to an increase in reflectance of 20% for kaolin-treated apple leaves. However, no effect on carbon assimilation was found with light levels over 1600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  as the light-saturation level was reached. At the whole-canopy level, the reduction in carbon assimilation rate was not detected. In addition, LeGrange et al. (2004) found a reduction in carbon assimilation of kaolin-treated apple leaves under light-limited conditions and mild temperatures. This was presumably due to an increase in light reflection and a decrease in light available to the chloroplasts. Rosati et al. (2006) confirmed this hypothesis in a recent study showing that kaolin reduced light-saturated CO<sub>2</sub> assimilation rate (by up to 4  $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ ) without any effect on stomatal conductance and increased intercellular CO<sub>2</sub> concentration in walnut and almond leaves one day after the kaolin application. The reduction in carbon assimilation by kaolin was minor compared to that of water-stress (Rosati et al. 2006). Rosati et al. (2006) modeled the photosynthetic response of kaolin-treated and untreated almond leaves and found that kaolin-treated leaves reached light saturation at higher PAR levels than control leaves. They concluded that kaolin reduced incident PAR by 37% on almond tree leaves due to shading effects. Leaf surface



characteristics among species and spraying techniques may explain the variation of PAR reduction found in the different studies.

In experiment 1, we measured a reduction of carbon assimilation from 2 to 7  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  between control leaves and kaolin-coated leaves. In experiment 2, we measured a reduction of 1.2 to 4.9  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  on the kaolin-treated second-leaf and a reduction from 2 to 3.1  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  on the third-leaf. Also, whole-canopy gas exchange studies in apple under mild temperatures have not shown reduction in global  $\text{CO}_2$  assimilation (Wünsche et al. 2004, Rosati et al. 2007). Kaolin film reduces carbon assimilation at the leaf-level but improves light distribution in the tree canopy increasing carbon assimilation at the tree-level, resulting in either negligible or positive effect of kaolin on yields. Similarly, we did not find significant differences in terms of cucumber growth (Table 3.3 and 3.6).

Three days after application, the second leaf recovered its photosynthetic abilities. In fact, the rapid growth of cucurbit leaves may explain that recovery. Leaf growth varied from 12 to 30  $\text{cm}^2$  a day (Table 3.7), therefore, the area covered by kaolin decreased over time. However, with multiple kaolin applications, we can hypothesize that the quality of kaolin coverage on the leaf remains fairly constant. This may explain why we measured lower carbon assimilation seven days after the second kaolin application on the second leaf (Table 3.4). The difference in response between the second and the third leaf may be also explained by leaf growth rate. The third leaf is larger and grows faster than the second leaf as shown in Table 3.7 and therefore returns to its normal photosynthetic rate faster.

In experiment 1, the third day after kaolin application, the kaolin-coated plants have a higher vapor pressure deficit (Figure 3.1) which explains why both stomatal conductance and transpiration are reduced since the plant has closed its stomates. However, the carbon assimilation is not affected because  $\text{CO}_2$  is taken from the intercellular spaces. The reason for the increase in vapor pressure deficit is still unknown as there was no difference in leaf temperature at the time of the reading.

Also, in experiment 1, we observed a reduction in gas exchange over time but this was not observed in experiment 2. These plants also flowered earlier than the controls

which might be a sign of competition for limited resources since cucumbers are not dependent on photoperiod or temperature to flower (Tesar 1984).

Kaolin's effect on plant growth was minimal. The majority of studies have reported either no or positive effects of kaolin film on plant productivity and yield of apple (Glenn et al. 2003; Garcia et al. 2004), pear (Puterka et al. 2000; Sugar et al. 2005), citrus (Jifon and Syvertsen 2003; Lapointe 2006), tomato (Makus 2000), pepper (Creamer et al. 2005; Russo and Diaz-Perez 2005), pecan (Lombardini et al. 2005) and blueberry (Spiers et al. 2003). However, some negative effects have been noted. Glenn et al. (2001a) found that apple yields were reduced with late applications of kaolin in temperate weather. Kaolin had negative effects on apple fruit size if applied after June (Schupp et al. 2002) and delayed fruit development of tomato (Makus 2000). After eight applications of kaolin, root dry weight of bean plants was reduced and shoot-to-root weight ratio was increased but there was no difference in leaf area (Tworkoski et al. 2002).

Based on our experiments we can conclude that kaolin had only a short term effect on carbon assimilation and negligible effects on transpiration and stomatal conductance and no significant effect on plant growth. Therefore it appears that cucumbers can be sprayed multiple times without long term negative consequences.

The effect on the plants of the old leaves coated with kaolin on physiological parameters of new leaves was not tested during the course of this experiment and remains to be tested. It would be interesting to study the plasticity of the plant responses.

## CHAPTER FOUR

### Effect of kaolin film on the behavior of the striped cucumber beetle *Acalymma vittatum* (Coleoptera: Chrysomelidae)

#### Introduction

Striped cucumber beetle (SCB), *Acalymma vittatum* (Fabricius) (Coleoptera: Chrysomelidae) is the main pest of cucurbit crops in northeastern America. A new non-toxic tool has been recently used for cucumber beetle control: kaolin clay marketed as Surround® WP crop protectant. Kaolin clay has been used successfully in other crops against a range of insects from a number of families (Glenn and Puterka 2005). The main mode of action of kaolin clay is the repellency of adults from the treated foliage. Kaolin clay interferes with the insects' host finding abilities. Consequently, feeding, oviposition, mating and survival may be reduced (Knight et al. 2000; Unruh et al. 2000; Cottrell et al. 2002; Puterka et al. 2005; Sackett et al. 2005). The modes of action differ according to the insect species and its behavior. *Acalymma vittatum* is a specialist herbivorous insect with good flying abilities. Its choice of host plant is determined by a series of visual, olfactory and tactile cues, either coming from the plants (Andersen and Metcalf 1986; Lewis et al. 1990; Hoffmann et al. 1996a) or from other individuals of *A. vittatum* (Smyth and Hoffmann 2003). To our knowledge, no studies have been carried out to elucidate the mechanisms by which *A. vittatum* is repelled from kaolin-coated plants. It is not known if the beetle is repelled before or after contact with the film or, indeed, if beetles fed on kaolin-treated leaves. Therefore, lab and field pilot experiments were designed to compare the preference and the behavior of the beetles given a choice between kaolin-treated and untreated plants and in a no-choice situation in the laboratory.

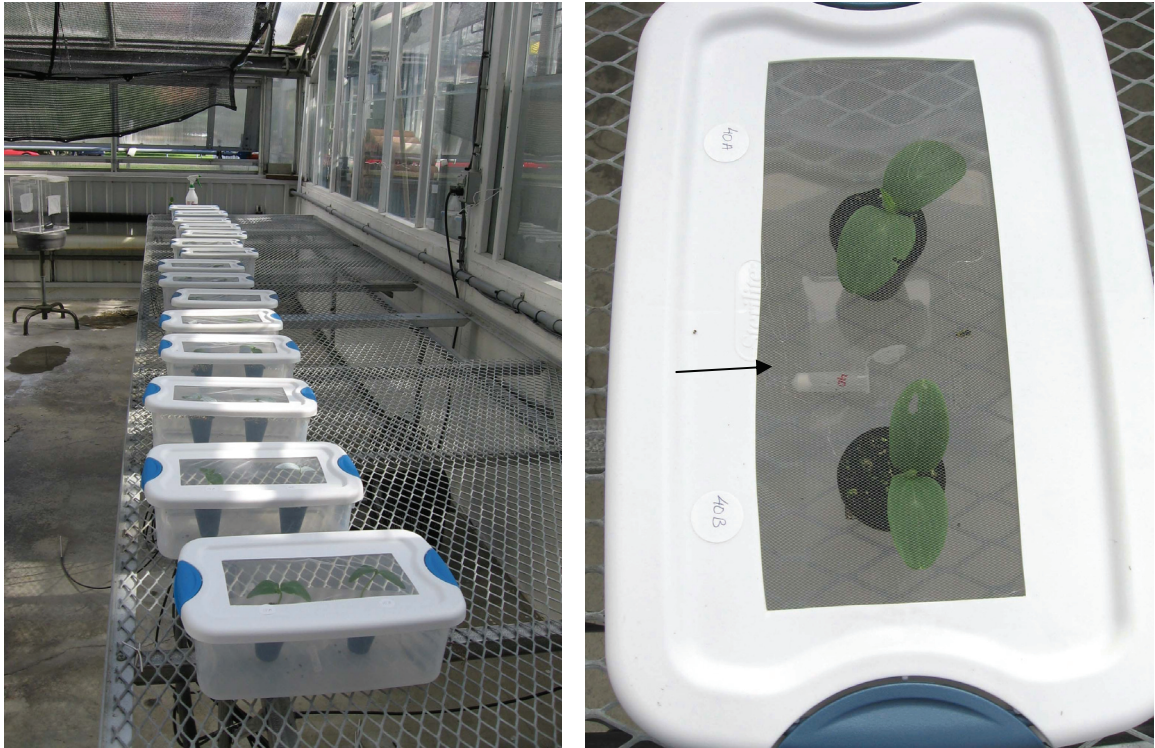
## Material and Methods

### *Experiment 1: Cage experiment*

The experiment compared three treatments: (1) no-choice situation with two untreated plants; (2) choice situation with one kaolin-treated plant and one untreated plant and (3) no-choice situation with two kaolin-treated plants. The experiment took place in a greenhouse on the Macdonald Campus of McGill University in Sainte-Anne-de-Bellevue, Québec (lat. 45° 26'N, long. 73° 56'W) in June 2006. The greenhouse was shaded with polypropylene mesh however, even with shading, day temperatures fluctuated between 22 and 30°C without additional light. Cucumbers cv. Speedway (Semences B.C., Laval, Québec) were sown in cylindrical 50 plugs trays (plug diameter 50 mm; ITML Horticultural Products inc., Brantford, Ontario) filled with Pro-mix BX (Premier Horticulture, Rivière-du-Loup, Québec). Plants were watered as required but not fertilized. Plants at the cotyledon stage were used in the experiment. Fifty grams of kaolin clay (Surround<sup>®</sup> WP; Engelhard corp., NJ, USA) was mixed per liter of water and sprayed on the top surface of the cotyledons with a hand-pump sprayer. About 50% of the leaf surface area was coated with kaolin clay (upper leaf surface).

SCB were collected from nearby organic cucurbit fields in Senneville (Québec, Canada) on June 4, 5, 16 and 20, 2006 with an aspirator container designed by Teshler et al. (2004) equipped with a water-saturated cotton wick. The beetles were stored in the container at 4°C and starved for 4 to 10 days prior to use. Teshler et al. (2004) found no effect on survivorship and behaviour of a similar species of beetles after being starved with this methodology.

Plastic boxes (46 x 31 x 19cm; Sterilite Corporation, Townsend, MA, USA) were used as cages (Figure 4.1). An observation/ventilation port was cut into the lid of the box and a light polyester mesh was glued over the port. Two plastic plugs were cut from the seeding trays and glued to the bottom of the cage equidistant to one another and to the sides of the box. Plants were randomly assigned to the cages and to one of the two plugs within each cage. Beetles were placed individually into a plastic test tube perforated to allow for air circulation (2ml G-Tube Flat-Top; Fisher Scientific, Ottawa, Ontario). Only one *A. vittatum* was used per cage to investigate behavior at the individual level.



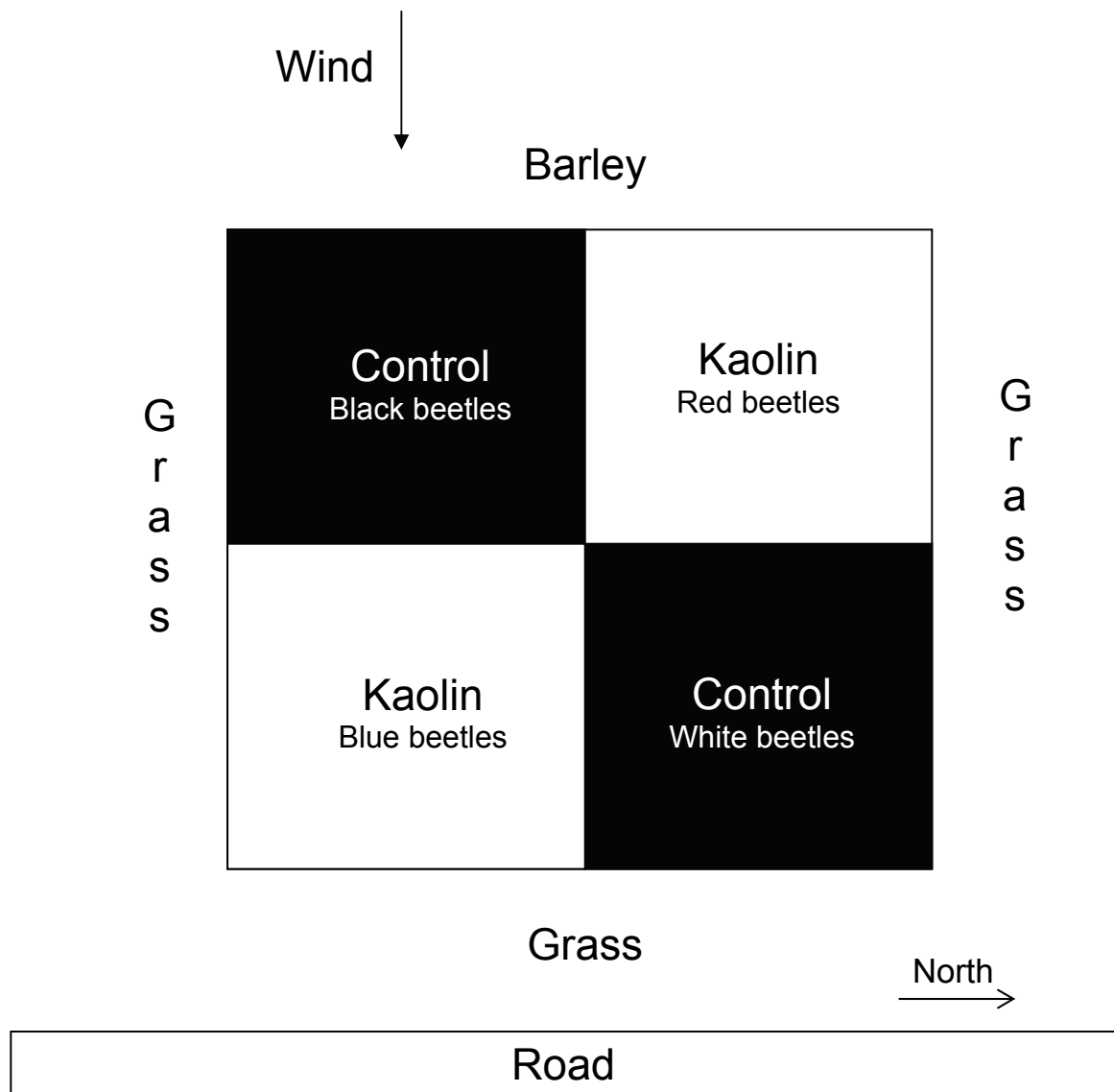
**Figure 4.1.** Cage experiment set-up and detailed view of the cage for the observation of *Acalymma vittatum* on cucumber cv. Speedway.  
The arrow indicates the test tube introduced to release the beetle.

Unsexed beetles were randomly assigned a cage and were released in the late afternoon at 16h30  $\pm$  30 min as recommended by Dernovici et al. (2006). The test tube was placed in the middle of the two plants on the floor of each cage and opened (Figure 4.1). The position of the beetle on the plants was recorded 15 minutes and 1 hour after placement and then at 8:30, 12:00 and 16:00h  $\pm$  30 min for the next 2 days. The treatments were replicated 10-15 times depending on the number of beetles available and the experiment was repeated three times for a total of 105 beetles. Uniform batch of beetles were used for each replicate (same day of capture and same period of starvation). After two days, beetles were removed of the cage, the location of damage recorded and the percent defoliation estimated visually by placing each plant in one of six defoliation classes: 0, 1-10, 11-25, 26-50, 51-75 and 76-100 % defoliation (adapted from Brewer et al. 1987).

**Statistical Analyses.** SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) was used. Data were subjected to Proc Freq and differences in the frequencies of beetles settling, location of settling and location of damage were determined with Pearson's chi-square test. For defoliation, classes were converted to averages of 0, 5.5, 18, 38, 63 and 88% defoliation and weighted average compared with tukey comparison test at  $P < 0.05$ .

### ***Experiment 2: Mark-release experiment***

A mark-release experiment comparing the behavior of *A. vittatum* in kaolin-treated and untreated cucumber plots was performed at Emile A. Lods Research Farm of Macdonald Campus, McGill University, Sainte-Anne-de-Bellevue, Québec (lat. 45° 25'N, long. 73° 55'W). The experimental field was chosen because no other cucurbit crops were being grown within a kilometer radius. The field was fall-ploughed, spring-harrowed and disked before the plastic mulch was laid. The field (15x12m) was surrounded by grass on three sides and barley on the windward side. It was divided into four sections (Figure 4.2) with each section having three mulched rows (Climagro, Plastitech, St-Rémi, Québec) spaced 1.5 m apart and 7.5 m in length. Cucumbers cv. Speedway (Semences B.C., Laval, Québec) were direct seeded 30 cm apart on June 27, 2006 through holes in the plastic mulch and watered via drip irrigation lines (T-Systems Inc., San Diego, CA, USA) under the mulch when necessary. Kaolin clay (Surround<sup>®</sup> WP, Engelhard corp., NJ, USA) was mixed with water (50 grams per liter) and sprayed on the



**Figure 4.2.** Field layout of the mark-release experiment of *Acalymma vittatum* painted with four different colors in a cucumber field cv. Speedway.

upper surface of the foliage using a 7.6 liter pump-sprayer (Thompson's Water Seal; Cleveland, Ohio, USA) six times during the course of the experiment. Applications were made July 5 (cotyledon stage), July 10 (first leaf stage), July 11 (first leaf stage), July 13 (second leaf stage), July 17 (fourth leaf stage) and July 18 (fourth leaf stage). Applications on July 11 and July 18 were necessary due to rain which had washed off the previous day's application (Appendix 3). Even with multiple applications, it was impossible to keep the foliage covered with kaolin at all times. Data presented are for the period from release until 6 days later (July 5 to July 11). *Acalymma vittatum* were collected from nearby organic cucurbit fields in Senneville (Québec, Canada) on June 22 to 28 with a multipurpose aspirator container equipped with a water-saturated cotton wick held at 4°C and starved until release following the method of Teshler et al. (2004). On July 3rd and 4th, four hundred beetles were marked on an elytron with Liquid Paper<sup>®</sup> correction fluid and once dry, were either left plain or colored with an Ultra-fine Sharpie<sup>®</sup> permanent color marker (to give four colors white, blue, red, and black) using the method of St-Pierre et al. (2005). One hundred beetles of the same color were placed in the multipurpose containers on the ground in the middle row of each of the four plots. The containers were shaded with plastic cup fixed over the container to prevent overheating and opened at 9 h a.m. on July 5 after the first kaolin application had dried on the foliage. Twenty randomly chosen plants were flagged in each plot to assess beetle presence and plant damage. The first 10 plants were located within a 2-meter diameter from the release point and the second 10 plants were located from 2 to 4 meters from the release point. The percent defoliation was estimated visually using six defoliation classes: 0, 1-10, 11-25, 26-50, 51-75 and 76-100 % (adapted from Brewer et al. 1987). Observations were made every two hours on the day of release, at 9:00, 13:00 and 17:00h the following day and then twice a day (9:00 and 17:00) for the 6 following days. Over the sampling period, 18 observations were done.

**Statistical Analyses.** Beetle retrieval rate was too low to conclude any significance between treatments. However, this pilot experiment gives us qualitative information about *A. vittatum* behaviour in contact with kaolin-treated plants. For defoliation, classes were converted to averages of 0, 5.5, 18, 38, 63 and 88% defoliation.



## Results

### *Experiment 1*

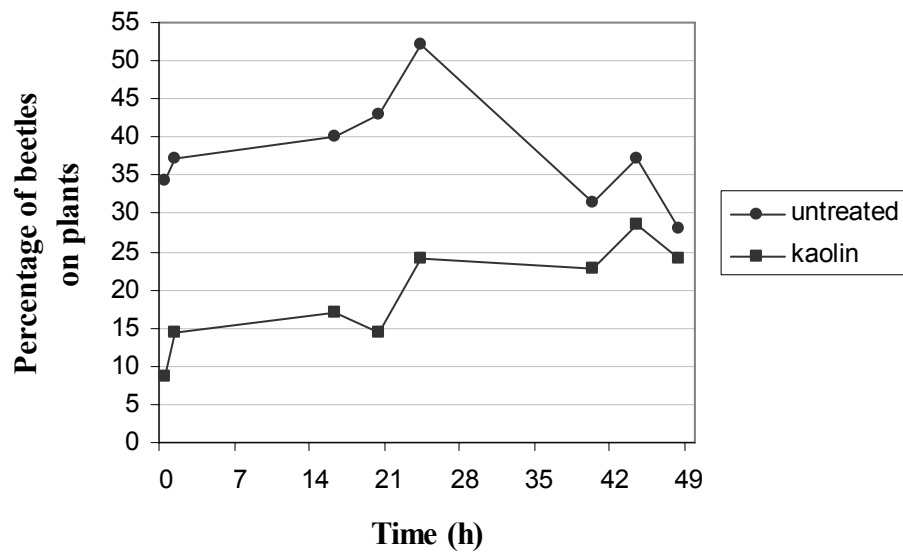
For the total of the eight sampling times, less than 30% of the introduced beetles were found on the cucumber plants in each of the three treatments (no-choice untreated, choice and no-choice kaolin). More than 50% of the beetles were still in the cage but not on the plants. Although the cages were sealed as tightly as possible between 14 to 19% of the beetles escaped in the course of the experiment. When comparing only the number of live insects within the cages, twice the number of beetles settled on untreated (48%) compared with kaolin-treated (24%) plants when given a choice (Table 4.1). In the case where there was no choice, the percentage of beetles settling was similar for both kaolin (35.9%) and untreated (34.3%) plants.

For the three treatments (no-choice untreated, choice, no-choice kaolin), 25-30% of the beetles had moved to the cucumber plants within an hour after release. The percentage of beetles settling on plants rose slowly, peaking after 20 hours for no-choice kaolin (38%) and after 24 hours for no-choice untreated and choice treatments (35% and 38% respectively). Forty-eight hours after release, the percentage of beetles on the plants decreased to 25-26% in all the treatments. When beetles had a choice, their first and sustained choice was the untreated plants (Figure 4.3). Thirty-seven percent of the beetles settled on untreated plants within 1 hour after release compared with 14% on the kaolin-treated plants. Within 24 hours, this had increased to 52% for the untreated and 24% for the kaolin and after 48 hours, this was 28 and 24% for the untreated and kaolin-treated plants, respectively.

On kaolin-treated plants, beetles settled primarily on the non-coated areas such as the lower leaf surface, the stem or the new growth for both choice and no-choice situations (Figure 4.4 A-B). This was even more apparent when beetles were given a choice (Figure 4.4-A).

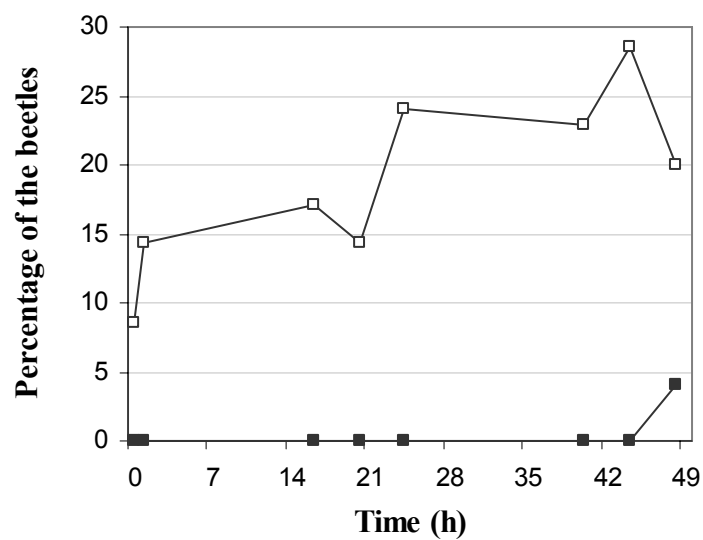
**Table 4.1** Effect of kaolin on the presence of *Acalymma vittatum* adults on foliage with or without a choice of kaolin-treated or untreated cucumber plants.

	Presence (%) of beetles settling		$\chi^2$	<i>P</i>
	Untreated	Kaolin		
No-choice	34.3	35.9	0.2804	0.60
Choice	48.0	24.0	25.5326	<0.01

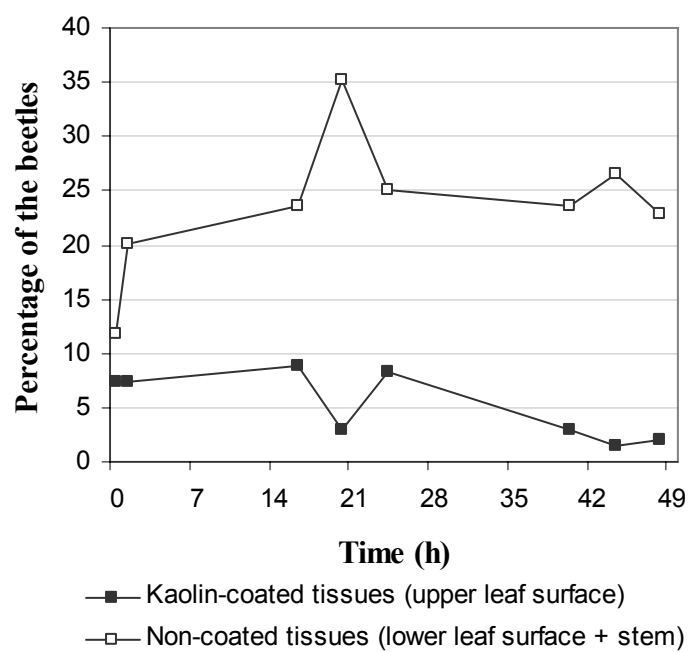


**Figure 4.3.** Percentage of striped cucumber beetle (*Acalymma vittatum*) located on either untreated or kaolin-treated cucumber plants in a choice situation over a 48 hour period after release (n=105 beetles).

### A. Choice



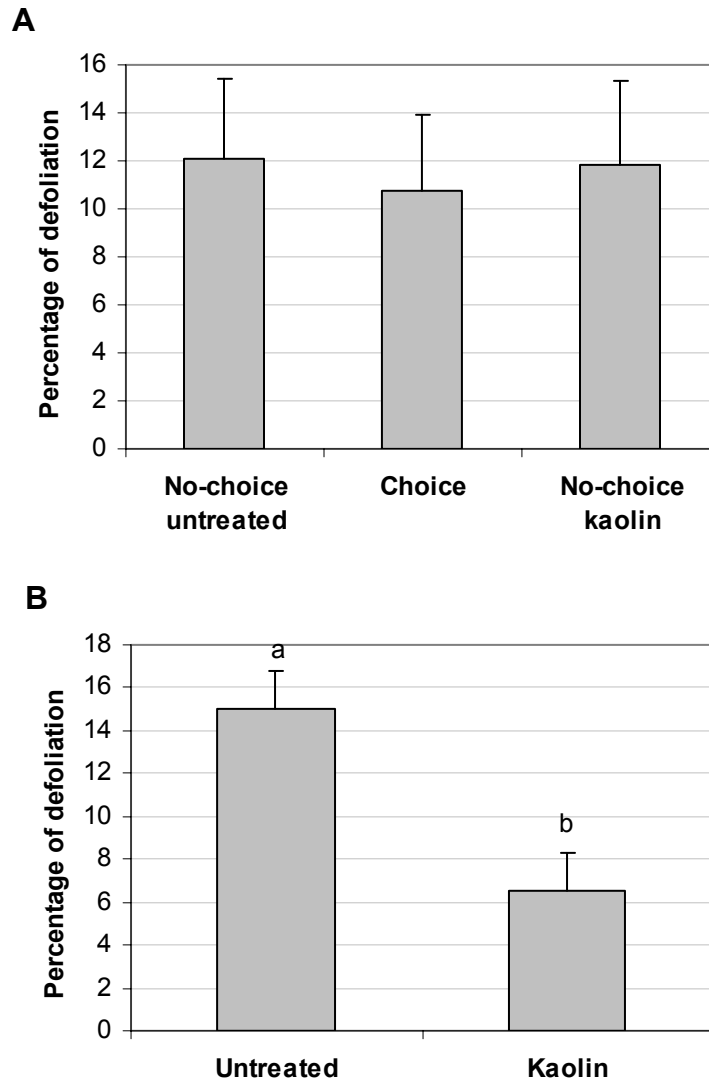
### B. No-choice



**Figure 4.4.** Effect of kaolin on location of *Acalymma vittatum* settlement on kaolin-treated cucumber plants in a choice (A) or no-choice (B) situation.

Defoliation did not differ among treatments with an average of 10 to 12 % defoliation (Figure 4.5-A). In the choice treatment, weighted mean defoliation was significantly greater on untreated plants (15% defoliation) than on kaolin-treated plants (6%; Figure 4.5-B). Percentages of damaged plants differed significantly between treatments in choice situations with 96% of the untreated plants damaged but only 62% of the kaolin-treated plants damaged ( $\chi^2=9.339$ ;  $P=0.002$ ). However, in no-choice situations, kaolin-treated plants (79%) were damaged similarly than untreated plants (75%) ( $\chi^2=0.2786$ ;  $P=0.5976$ ).

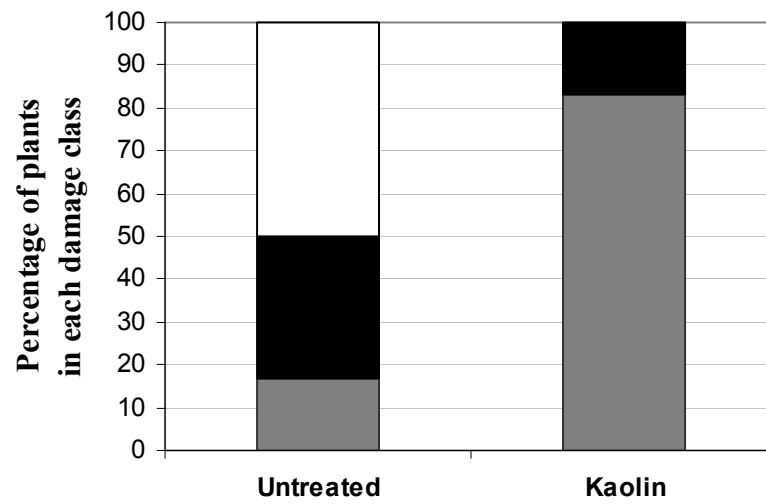
Beetles fed less on the upper leaf surface of plants that was treated with kaolin compared with untreated plants in both choice and no-choice situations (Figure 4.6 A-B). In choice cages, 83% of the untreated plants were damaged on the upper leaf surface and only 17% of the kaolin treated plants were damaged on the upper leaf surface ( $\chi^2=13.03$ ;  $P<0.01$ ; Figure 4.6-A). In no-choice cages, 86% of the untreated plants were damaged on the upper leaf surface and 30% for the kaolin treated plants ( $\chi^2=5.57$ ;  $P=0.02$ ; Figure 4.6-B). The beetles still feed on kaolin-treated plants however; they feed primarily in the uncoated areas.



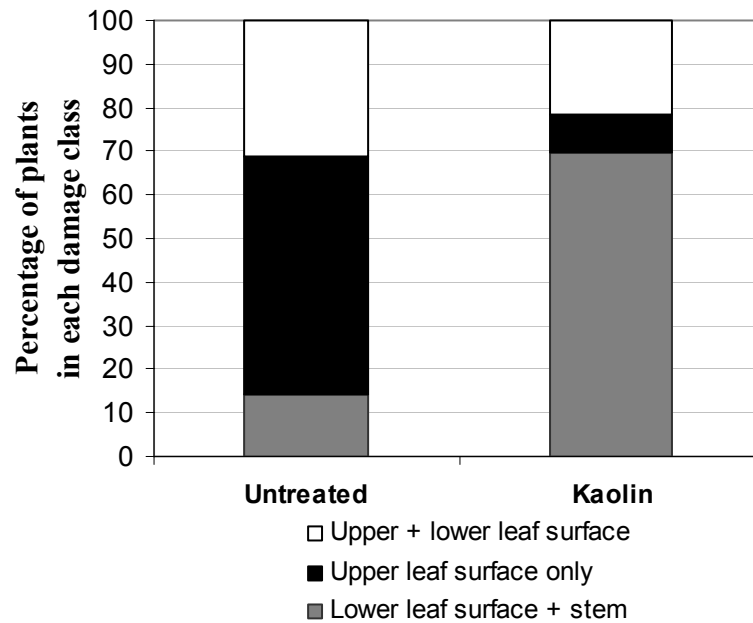
**Figure 4.5.** Percentage of defoliation by *Acalymma vittatum* of the cucumber plants for the three treatments (no-choice untreated, choice or no-choice kaolin) (A) and within a choice situation (B).

Percentage of defoliation is the weighted average of each defoliation category compared with tukey multiple comparison test at  $P < 0.05$ .

### A. Choice



### B. No-choice



**Figure 4.6.** Effect of kaolin-treated cucumber plants compared to untreated on the location of damage by *Acalymma vittatum* with choice (A) and without choice (B).

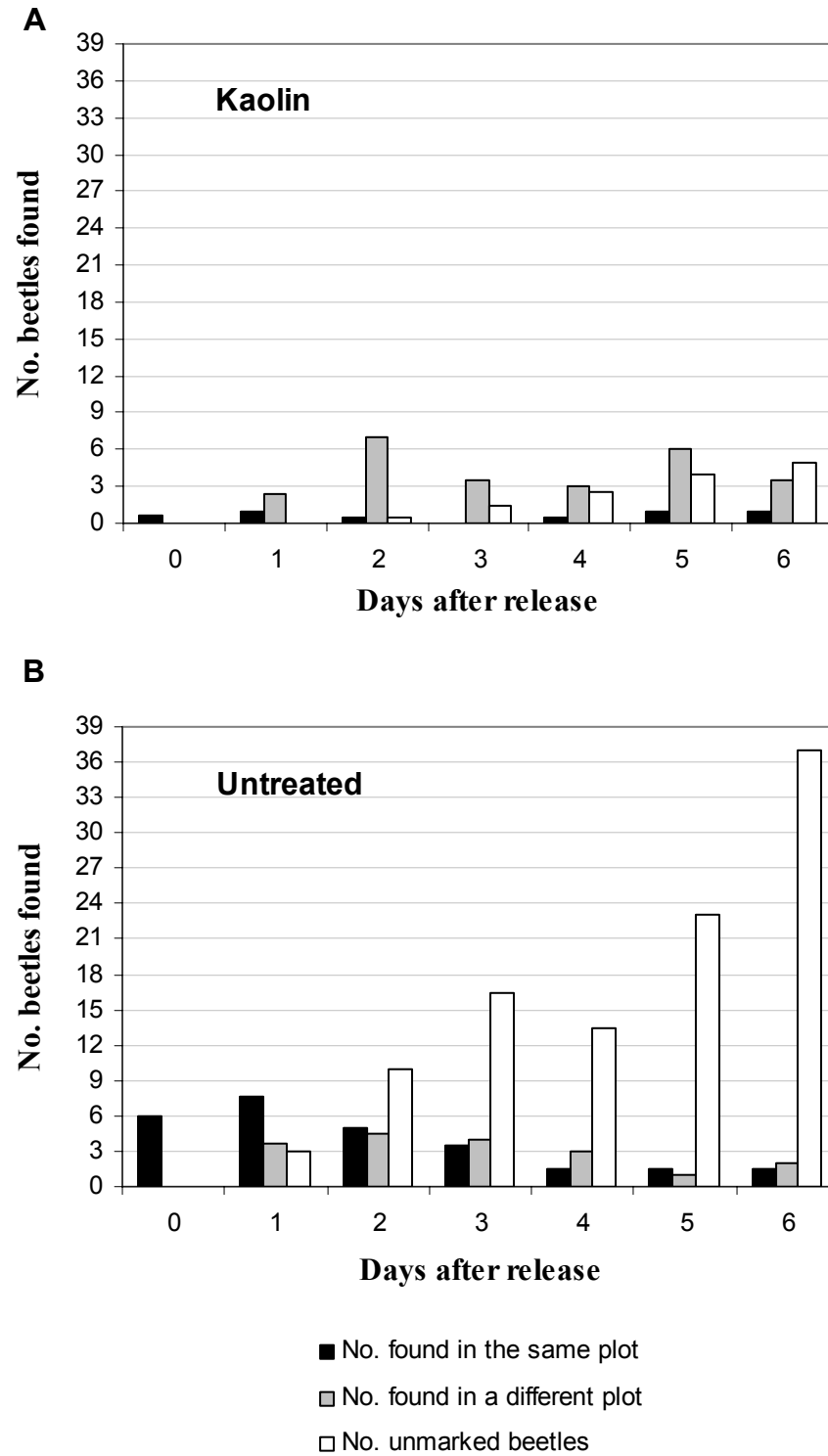
## ***Experiment 2***

Out of a total of 400 marked individuals, 18 (4.5%) were found in either the untreated or kaolin-treated plots 48 hours after release, when 20 of the 75 plants in each experimental plot were sampled. Marked beetles were counted repetitively on the same plants as beetles were not recaptured. In a similar study, Bach (1980b) retrieved 11.5% of the released *A. vittatum* when she sampled all the plants in an experimental plot (81-84 plants per plot). In an ecological study examining the dispersal ability of chrysomelid beetles, a retrieval rate of 13 to 16% was reported (St-Pierre et al. 2005). In our study, winds (17 to 30 km/h) on the day of release may have encouraged dispersal of the beetles outside of the experimental plots. The low retrieval rate and the low number of repetitions unable the use of statistics; however, this pilot experiment gives us qualitative information about *A. vittatum* behaviour in contact with kaolin-treated plants.

On the day of release, beetles were seen exclusively in the plot into which they were released (Figure 4.7). The following day, they were also seen in other plots. However, beetles released in untreated plots tended to remain in the same untreated plot (Figure 4.7 B). Forty-eight hours after release, only 1 of the beetles released into the kaolin plots was still there and none had move to the other kaolin-treated plot, 7 beetles had moved to an untreated plot and 1 unmarked beetle (new beetle) had arrived (Figure 4.7 A). On the other hand, for the untreated plots, 6 beetles remained where they had been released, 4 beetles had moved in equal proportions to other untreated or kaolin plots and 13 unmarked beetles had arrived. A similar pattern was seen in the following days but over time the number of marked beetles decreased and unmarked beetles increased. Unmarked beetles started to migrate into untreated plots one day after release, attracted either by the cotyledon stage plants and/or the aggregation pheromone secreted by beetles feeding on the plants (Smyth and Hoffmann 2003). The unmarked beetles migrated primarily to untreated plots throughout the observation period (Figure 4.7).

When comparing the number of times that beetles were counted during the 18 observations over the 6 day period, fewer marked beetles were found in kaolin (65) than in untreated plots (119). Fewer beetles over all were found in kaolin (12) versus untreated plots into which they were released (79). More beetles from kaolin-treated plots were





**Figure 4.7.** Number of marked and unmarked *Acalymma vittatum* found, released in kaolin (A) and untreated (B) cucumber plots.

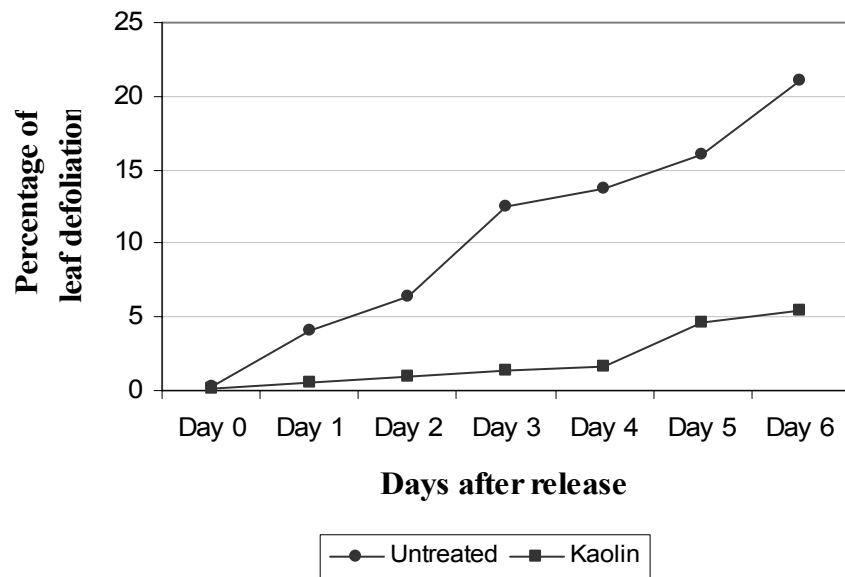
Numbers shown are the mean sampling counts for each day (day 0 n=5; day 1 n=3; day 2-6 n=2). The data from the two plots of each treatment are combined.

later found in another plot (53) than beetles from untreated plots (40). More beetles from kaolin plots were later found in untreated plots than in the other kaolin plot.

Defoliation was lower in kaolin-treated plots compared with the untreated control (Figure 4.8). The mean percentage defoliation over the entire plot was 20% for untreated plants compared with 5% for kaolin-treated plants six days after the insects were released. At that time the plants had reached the second leaf stage and such a loss of 20% of the foliage was significant. Early defoliation causes slower growth rate, delays flower production (Brewer et al. 1987) and reduces yields (Burkness and Hutchison 1998). The large number of unmarked beetles migrating into the untreated plots was unexpected due in part to the distance from neighboring cucurbit crops (>1 km) and the date (July). However, further experiments are needed to support the results because of the low sample size of this pilot experiment.

## Discussion

Our results showed a reduction of feeding damage and settling on kaolin-treated plants in choice situation but not in no-choice situations. Our results are consistent with a study evaluating the defoliation by *Plutella xylostella* larvae (Lepidoptera) in no-choice with untreated or kaolin-treated plants when untreated plants had only slightly larger areas consumed (Barker et al. 2006). However, Lapointe (2000) working with the adult root weevil *Diaprepes abbreviatus* (Coleoptera: Curculionidae) observed that kaolin reduced feeding damage, settling and oviposition on the citrus leaves in choice and no-choice situations. The beetles used for that experiment were reared and not starved prior to use. However, our beetles were starved for 4 to 10 days before the experiment which presumably increased their voracity. *A. vittatum* settled and fed mainly on non-coated areas of the kaolin-treated plants (less than 10% of the beetles settled on coated areas and 30% of the plants had damage on the treated upper leaf surface; Figure 4.4 and 4.6). Further research is needed to investigate effect of kaolin feeding behaviour of *A. vittatum* and the possible impact on pest-parasitoid interaction. Also, field experiments in no-choice situations would be necessary to test if *A. vittatum* really fed on treated areas under field conditions.



**Figure 4.8.** The mean percentage of leaf defoliation caused by *Acalymma vittatum* on untreated and kaolin-treated cucumber plants during a period of 6 days after the insects were released.

In the field, the preliminary results of the mark-release experiment show that *A. vittatum* released in untreated plots stayed longer in the same plot than in kaolin-treated plots. Bach (1979) found that marked *A. vittatum* stayed in the same area after release in cucumber monoculture, meaning that the natural habit of the beetle is not to migrate when a suitable host plant is available. The movement pattern of *A. vittatum* in this context was characterized by greater immigration into untreated plots and longer tenure time in those plots which resulted in a greater number of beetles. The beetles avoided the kaolin-treated plots both by greater emigration and by less immigration.

The effects of kaolin depend on the insect species and are specific to the insect behavior and biology. The main action of kaolin found was to deter insect settling and oviposition (Glenn et al. 1999; Lapointe 2000; Puterka et al. 2000; Unruh et al. 2000; Showler 2002a; Puterka et al. 2003; Sisterson et al. 2003; Showler 2003; Sackett et al. 2005). Reduced survival of adult and larvae was recorded on kaolin-treated foliage (Knight et al. 2000; Unruh et al. 2000; Cottrell et al. 2002; Showler 2003; Puterka et al. 2005; Sackett et al. 2005). In addition, there was a reduction in the mating of lepidopterans exposed to kaolin (Knight et al. 2000; Puterka et al. 2005). Puterka et al. (2000) hypothesized that kaolin may act by creating interference with the insect's tactile perception of the host plant for pear psylla (*Cacopsylla pyricola*; Homoptera). Our study showed that *A. vittatum* moved first toward untreated plants when given a choice. This demonstrates that kaolin is affecting the beetle at a distance rather than being limited to a contact effect. In a similar experiment on *Plutella xylostella* (Lepidoptera) in choice tests with kaolin and untreated plants, it was observed that the larvae first moved toward the untreated leaves (Barker et al. 2006). Showler (2002a) also showed that kaolin affected the insect at a distance rather than being limited to direct contact. Kaolin has a deterrent effect against *A. vittatum* that could be either visual or olfactory. We know that *Diabrotica* spp. are sensitive to yellow-green and near-ultraviolet spectra (Agee et al. 1983). The color change of the foliage either to a white, grayish or mottled pale green compared with the untreated bright green may mean that the cucumber beetles are less able to recognize their hosts. Further studies would be necessary to confirm which cues are affected.

This pilot study did not answer the question of survivorship of *A. vittatum* on the kaolin-treated plants and the effect of direct application of kaolin on *A. vittatum* adult is unknown. Although in the greenhouse experiments there was no significant difference in survivorship, the number of beetles observed in the kaolin field was too small to make any conclusion. It is equally possible that the beetles escaped from the experimental field, looking for a better host (even if no host was available within a 1 km distance) or died. It is unknown if kaolin has a negative effect on the performance of *A. vittatum*. Life table studies would help to identify whether kaolin affects mating success, oviposition, larval development and overall development time.

The present studies suggest that kaolin affects *A. vittatum* under laboratory and field conditions by creating a physical barrier to host plant. Kaolin can be used as an alternative to insecticides to deter *A. vittatum* and reduce feeding damage and bacterial wilt (*Erwinia tracheiphila*) transmission especially for seedlings and young plants. However, non-covered plant tissues are still susceptible to attack by SCB. Therefore, it is necessary to develop a method that maintains overall coverage which takes into account both the rapid growth of the cucurbit crop and the beetle attraction to the non-covered portion of the plant.

## **CHAPTER FIVE**

### **Conclusions and summary**

This project had the objectives to determine the efficacy of kaolin in reducing the striped cucumber beetle (SCB) population, damage, bacterial wilt infection and cucumber yields compared to conventional practices in field conditions. The second objective was to evaluate the impact of kaolin coating on the leaf gas exchange and growth of cucumber plants, and finally to investigate the behaviour of SCB in contact with kaolin.

In field trials, kaolin was effective in reducing SCB populations, damage, bacterial wilt and enhanced yields in 2005 under a maximum beetle population of 0.2 beetles per plant before harvest in kaolin. However, in 2006, kaolin reduced beetle population and damage up to the fourth leaf stage but population reached 4.3 beetles per plant before harvest in the kaolin treatment. Beetle seasonal mean, bacterial wilt incidence and yields were not different from untreated controls in 2006. In greenhouse trials, single and multiple applications of kaolin had negative short term effects on cucumber leaf carbon assimilation but only negligible effects on transpiration, stomatal conductance and plant growth. Laboratory and field behavior studies showed that SCB has a preference for non-treated plants with reduced settling frequency and damage on kaolin-treated plants. Maintaining complete kaolin coverage is difficult and SCB can settle and feed on non-treated plant surfaces. SCB feeding behavior in no-choice conditions is still uncertain because 30% of the kaolin-treated plants were damaged on treated surfaces in the cage experiment. No-choice situation with only kaolin-treated plants was not tested in the field.

Kaolin still shows potential to be included in cucurbit production especially to control the overwintering beetles which damage young plants. Special attention must be taken in fields with bacterial wilt problems. Beetle monitoring is important and SCB populations must be kept under thresholds of 0.5-1 beetle per plant under five leaves and 3-5 beetles per plant over five leaves in order to prevent yield losses. Complementary control methods can be used to reduce beetle population if it exceeds the thresholds. In that case, reduced risk insecticides could be applied to specific field areas or to the entire field.

### **Further research**

- Study in depth the efficacy, the pesticide reduction and the costs of kaolin use and striped cucumber beetle control strategies in conventional and organic cucurbit fields in a large scale study.
- Test the combination of kaolin and other alternative control methods such as entomopathogenic nematodes and fungus, kairomonal baits and trap crops.
- To develop a successful IPM program, continue testing the efficacy of reduced risk and organic insecticides such as spinosad.
- Study the effect of kaolin on survivorship, mating success, oviposition, larval development and development time of *Acalymma vittatum* with life table studies.
- Effect of kaolin on host plant finding cues such as plant olfactory signals and colour change.

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## APPENDIX 1

**Table A.1.** Average temperature (°C) from May to August in Dorval and Sainte-Anne-de-Bellevue for the 2005 and 2006 seasons.

Year	May	June	July	August	Season-mean
2005 *	11.9	21.5	22.2	21.7	19.3
2005 †	11.5	20.9(E)	21.6(E)	21	21.2(E)
2006 *	14.5	19.2	22.6	19.3	18.9
2006 †	14.2	18.7	22.2	19.1(E)	18.6(E)
Avg 1977-2000*	13.4	18.2	20.9	19.6	18.03

\*Montréal Pierre-Elliott Trudeau International Airport station, Dorval, Québec (at 20 km from experimental site; lat. 45°28'N, long. 73°45'W; Environment Canada).

†Sainte-Anne-de-Bellevue station, Québec, (lat. 45°25'N, long. 73°55'W ; Environment Canada).

E= estimated

**Table A.2.** Total precipitation (mm) from May to August in Dorval for the 2005 and 2006 seasons.

Year	May	June	July	August	Season-total
2005	43.0	129.0	125.6	134.0	431.6
2006	173.4	104.2	135.2	154.4	567.2
Avg 1977-2000	76.3	83.1	90.3	97.7	343.4

Montréal Pierre-Elliott Trudeau International Airport station, Dorval, Québec (at 20 km from experimental site; lat. 45°28'N, long. 73°45'W; Environment Canada).

## APPENDIX 2

**Table A.3.** Daily meteorological data for Montréal Pierre-Elliott Trudeau International Airport station (lat. 45°28'N, long. 73°45'W), Dorval, Québec for the 2005 season (Environment Canada).

Month	Day	Max temperature	Min temperature	Mean temperature	Total precipitations
		°C	°C	°C	mm
May	1	13.2	5.8	9.5	0.4
May	2	10.7	4.6	7.7	2.4
May	3	11.2	3.6	7.4	0.2
May	4	11	4.1	7.6	0
May	5	14.9	2.2	8.6	0
May	6	19.9	5	12.5	0
May	7	17.3	7.6	12.5	0
May	8	16	7.2	11.6	0
May	9	19.8	9.1	14.5	0
May	10	27	10.4	18.7	0
May	11	25.9	6.5	16.2	1.4
May	12	11.1	0.8	6	0
May	13	14.3	-1.2	6.6	0
May	14	11.2	8.4	9.8	2.6
May	15	17.3	7.2	12.3	6.6
May	16	17.1	9.4	13.3	1.6
May	17	14.6	6.2	10.4	0
May	18	14.6	5.4	10	0.2
May	19	17.7	3.4	10.6	0
May	20	19.3	7.3	13.3	T
May	21	13.2	8.8	11	T
May	22	10.9	8.2	9.6	2.8
May	23	12.6	8.2	10.4	7.6
May	24	18.5	8.8	13.7	0
May	25	21.3	8.4	14.9	0
May	26	14.1	9.1	11.6	1
May	27	13.1	10.2	11.7	9.6
May	28	22.6	10.8	16.7	0.2
May	29	22	8.2	15.1	T
May	30	20.7	12.5	16.6	6.4
May	31	23.5	12.8	18.2	0
June	1	26.1	11.7	18.9	0
June	2	28	12	20	0
June	3	27.9	14.7	21.3	0
June	4	30.1	17.1	23.6	0
June	5	27.6	16.6	22.1	0
June	6	30.8	13.5	22.2	4.6
June	7	27.5	16.2	21.9	0
June	8	18.7	13	15.9	0.4
June	9	29.7	13.8	21.8	0
June	10	29.7	18.8	24.3	1.2



June	11	32.7	22.1	27.4	0
June	12	32.2	22.6	27.4	T
June	13	31.2	22.9	27.1	T
June	14	25.2	16	20.6	33.2
June	15	20	15.8	17.9	36.6
June	16	20.3	14.3	17.3	5.8
June	17	15.7	11	13.4	45.6
June	18	16	10.9	13.5	T
June	19	21.6	11.8	16.7	0
June	20	25	12.2	18.6	0
June	21	27.5	16.4	22	0.8
June	22	22.5	12.8	17.7	0
June	23	21.9	8.8	15.4	0.6
June	24	29.9	16.2	23.1	0
June	25	32.6	21.7	27.2	0.2
June	26	30.4	18.9	24.7	0
June	27	32.5	18.4	25.5	0
June	28	32.6	21.9	27.3	0
June	29	27.8	22.9	25.4	T
June	30	29.1	20.7	24.9	0
July	1	30.4	19.9	25.2	6.4
July	2	23.1	14.6	18.9	0
July	3	27.2	14.4	20.8	0
July	4	29.6	16.6	23.1	0
July	5	27.1	18.8	23	63.6
July	6	23.4	16.6	20	0
July	7	25.3	13.6	19.5	0
July	8	25.5	16.2	20.9	T
July	9	20.6	16	18.3	20.6
July	10	31.5	17.2	24.4	0
July	11	32.8	20.2	26.5	0
July	12	30.6	19.2	24.9	0
July	13	26.8	19.2	23	0.2
July	14	29.2	18.8	24	0.2
July	15	28.9	18.1	23.5	0
July	16	31.2	17.5	24.4	0
July	17	27.8	23.4	25.6	8.6
July	18	31	24	27.5	T
July	19	31.1	22.5	26.8	T
July	20	26.8	18.7	22.8	0
July	21	30.6	19.3	25	0
July	22	27.1	15.3	21.2	15.8
July	23	25.9	15.1	20.5	0
July	24	24.6	11.8	18.2	0
July	25	31	20.7	25.9	2.4
July	26	26.6	18.9	22.8	5.6
July	27	21.5	11.8	16.7	2.2
July	28	23.2	9.9	16.6	0
July	29	25.3	14.1	19.7	0
July	30	24.7	12.4	18.6	0

July	31	24.8	14.3	19.6	0
August	1	27	17.8	22.4	0.2
August	2	31	19.9	25.5	6.4
August	3	28.2	17.6	22.9	0
August	4	32.1	20	26.1	6.4
August	5	28.3	18.2	23.3	9.2
August	6	25.4	14.5	20	0
August	7	28.6	16.5	22.6	0
August	8	30.4	19.5	25	0
August	9	30.9	19.9	25.4	0
August	10	30.6	20.1	25.4	3
August	11	28.5	19.8	24.2	0
August	12	26.7	18	22.4	3
August	13	28.1	19.5	23.8	0
August	14	23.8	17.8	20.8	0.8
August	15	27	15.4	21.2	0
August	16	28	18.4	23.2	T
August	17	23.5	10.9	17.2	0
August	18	20.6	8.1	14.4	0
August	19	23.2	14.9	19.1	8
August	20	22.4	18	20.2	4.8
August	21	27.4	17.7	22.6	6
August	22	23.6	14.2	18.9	0
August	23	22.1	13.1	17.6	T
August	24	21.8	11.4	16.6	T
August	25	27.6	12	19.8	0
August	26	28.2	16.3	22.3	0
August	27	29.5	13.8	21.7	T
August	28	27.8	18.5	23.2	12.4
August	29	28.2	17.3	22.8	0
August	30	27.9	18.6	23.3	T
August	31	22.2	17.8	20	73.8

T= Trace

### APPENDIX 3

**Table A.4.** Daily meteorological data for Montréal Pierre-Elliott Trudeau International Airport station (lat. 45°28'N, long. 73°45'W), Dorval, Québec, 2006 (Environment Canada).

Month	Day	Max temperature °C	Min temperature °C	Mean temperature °C	Total precipitations mm
May	1	22.8	8	15.4	T
May	2	15	8.2	11.6	19.6
May	3	12.8	8.8	10.8	3.6
May	4	24	5.4	14.7	0.2
May	5	19.9	9.3	14.6	0
May	6	12.8	1.2	7	7.6
May	7	13.6	-0.2	6.7	0
May	8	21.8	4.5	13.2	0
May	9	23	10.6	16.8	0
May	10	20	11.8	15.9	0
May	11	23.8	11.4	17.6	0
May	12	18.4	13.6	16	8.6
May	13	13.6	10.5	12.1	15.6
May	14	21.2	10.6	15.9	2
May	15	19.6	10.9	15.3	5.6
May	16	16.8	11.3	14.1	14.6
May	17	14.7	11.6	13.2	4.6
May	18	16.6	9.8	13.2	6
May	19	11.8	9	10.4	45.6
May	20	11.3	7.8	9.6	6.2
May	21	13.5	7.6	10.6	7.8
May	22	9.9	4.5	7.2	0.6
May	23	15.6	6	10.8	T
May	24	20	7	13.5	0
May	25	25.6	8.8	17.2	0
May	26	22.4	15.3	18.9	1.8
May	27	22.5	14.7	18.6	0
May	28	27.1	12.7	19.9	0
May	29	28.1	18.2	23.2	0
May	30	25.9	17.3	21.6	T
May	31	30.8	16.3	23.6	23.4
June	1	22.4	12.9	17.7	6.2
June	2	25.4	10.5	18	0
June	3	17.5	13.6	15.6	9.6
June	4	20.4	12	16.2	0
June	5	24.9	8.9	16.9	0
June	6	27.7	14	20.9	0
June	7	26.5	15.3	20.9	T
June	8	17.5	12.1	14.8	0.8
June	9	16.6	12	14.3	6.4
June	10	13.8	8.9	11.4	25
June	11	20	8.8	14.4	4.8

June	12	21.1	13.7	17.4	0
June	13	23.2	13.8	18.5	T
June	14	24.7	14.4	19.6	0
June	15	25	11.1	18.1	T
June	16	29.1	15.2	22.2	0
June	17	25.5	17.5	21.5	0.2
June	18	32.4	18.6	25.5	0
June	19	28.1	18.9	23.5	1.2
June	20	23.5	13.1	18.3	8.4
June	21	24.2	10.7	17.5	0
June	22	27.6	18.4	23	0.8
June	23	22.9	16.4	19.7	0
June	24	24.8	13.4	19.1	0
June	25	27.7	13.5	20.6	0.2
June	26	23.5	20	21.8	1.8
June	27	27.9	20.2	24.1	14.4
June	28	25.2	19.9	22.6	9
June	29	27.8	17.5	22.7	0
June	30	21.7	15	18.4	15.4
July	1	25.6	15.2	20.4	3.8
July	2	29.9	15.3	22.6	19.4
July	3	26.8	13	19.9	1.4
July	4	27.8	17.7	22.8	18.8
July	5	23.4	14.4	18.9	1
July	6	23.9	12.9	18.4	0
July	7	27.1	15.9	21.5	0
July	8	28.2	17.8	23	0
July	9	28.1	16.6	22.4	0
July	10	29.2	18.6	23.9	6.2
July	11	26.8	19	22.9	0
July	12	25.1	20	22.6	0.2
July	13	29.9	18.5	24.2	0
July	14	31.5	21.3	26.4	0
July	15	27.4	21	24.2	3
July	16	30.8	19.5	25.2	0
July	17	32.3	19.3	25.8	6.8
July	18	27.6	16.1	21.9	1
July	19	28	14.2	21.1	0
July	20	30.6	18	24.3	14.6
July	21	27.5	20	23.8	1.4
July	22	22.7	17.2	20	3.8
July	23	25.4	16.3	20.9	1.6
July	24	25.8	17.2	21.5	T
July	25	24.5	18.7	21.6	35
July	26	28.7	19.6	24.2	T
July	27	29.7	22.6	26.2	1
July	28	26.2	21.4	23.8	0
July	29	29.7	18.5	24.1	T
July	30	24	14.4	19.2	0
July	31	27	17.3	22.2	16.2

August	1	34	22.6	28.3	19.4
August	2	29.6	18.3	24	48.2
August	3	27.1	17.7	22.4	4.2
August	4	29.4	16.8	23.1	1.4
August	5	23.9	12.9	18.4	0
August	6	26.6	14.6	20.6	0
August	7	28.8	18.6	23.7	0.8
August	8	25	13.7	19.4	0
August	9	25.5	12.7	19.1	0
August	10	21.8	14.5	18.2	5.2
August	11	21.1	10.6	15.9	T
August	12	21.8	9.3	15.6	0
August	13	23.4	10.9	17.2	0
August	14	25	15.2	20.1	3.2
August	15	25.1	17.9	21.5	T
August	16	25.3	14.6	20	0
August	17	25.9	13.4	19.7	0
August	18	29.9	17.2	23.6	0
August	19	29.1	19.8	24.5	18.4
August	20	20.2	15.2	17.7	13.4
August	21	25.4	15.6	20.5	2.4
August	22	25.1	14.2	19.7	0
August	23	20.5	11.1	15.8	0
August	24	21.1	9.2	15.2	0.6
August	25	20.1	9.8	15	0
August	26	22.9	8.9	15.9	0
August	27	17.9	14.5	16.2	37.2
August	28	22	14.7	18.4	T
August	29	24.4	15.2	19.8	T
August	30	20.2	10.2	15.2	0
August	31	20.8	7.9	14.4	0

T= Trace