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# ECOLOGICAL WINDOWS FOR STABLE TRITROPHIC INTERACTIONS IN AGRO-ECOSYSTEMS

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## RÉSUMÉ

Le sujet de cette thèse concerne les effets de la résistance des plantes sur l'abondance d'un insecte ravageur et les conséquences de cette variation sur la survie des stades juvéniles d'un prédateur de ce ravageur. L'hypothèse qui fut testée était que la survie augmenterait avec une diminution de la résistance, puisque cela permettrait une abondance plus grande de proies, mais que l'on ne devrait pas observer une augmentation de la survie passé un certain seuil de proies. Cette hypothèse fut testée dans un agro-écosystème édifié à partir de génotypes de maïs différentiellement résistants, le composé secondaire, DIMBOA, étant le facteur de résistance, des pucerons de l'espèce *Rhopalosiphum maidis* (Homoptera: Aphididae) comme ravageurs, et des coccinelles (Coleoptera: Coccinellidae) comme prédateurs. Parce que les facteurs de résistance chez les plantes sont souvent tributaires des conditions environnementales, il fut également décidé de déterminer quels seraient les effets du stress chez le maïs sur la survie des coccinelles. Dans cet objectif, les plants expérimentaux ont été soumis à des variations soit de densité, soit de niveau de fertilisation.

Une expérience préliminaire a permis d'établir que le développement des coccinelles de l'espèce *Propylea quatuordecimpunctata* n'était pas affecté lorsque les larves étaient nourries avec des pucerons reproduits à partir d'un génotype à contenu élevé en DIMBOA. Cependant, une augmentation significative d'environ une demi journée a été observée dans le temps de pupaison chez les coccinelles nourries avec des pucerons reproduits à partir de plants stressés du même génotype (Chapitre III). En revanche, les coccinelles adultes ont démontré de façon significative une préférence pour des plants préalablement stressés en serre et par la

suite transférés en champ, alors que ces plants n'étaient pas infestés par des pucerons. Cette préférence était indépendante du génotype (Chapitre IV). Cependant, lorsque les plants étaient naturellement infestés de pucerons, la réponse reproductive des coccinelles était reliée au nombre maximal de pucerons observés, et non pas à l'apparence des plants lorsque ceux-ci étaient soumis à un faible niveau de fertilisation, par exemple (Chapitre V).

Dans une autre expérience, réalisée en serre avec des génotypes à contenu en DIMBOA faible, intermédiaire et élevé, une augmentation de plus du double dans la concentration du métabolite a été observée chez des plants stressés du dernier génotype, alors qu'aucune variation due au stress n'a été observée chez les autres génotypes (Chapitre II). Cependant, l'augmentation de la concentration en DIMBOA chez les plants stressés du génotype élevé ne s'est pas traduit par une augmentation de la résistance contre le puceron *R. maidis* puisque les plants de ce génotype se sont révélés déjà très résistants. Une diminution de la croissance des colonies de pucerons a pu, en revanche, être observée sur des plants stressés du génotype faible en DIMBOA. Ces résultats sont similaires aux tendances observées en champ (Chapitre V). Ainsi, il est possible de conclure que le déploiement de variétés de maïs contenant des concentrations intermédiaires ou élevées de DIMBOA seraient des stratégies durables contre le puceron *R. maidis* dans ce système.

En champ, la réponse reproductive des coccinelles, en relation avec le nombre maximal de pucerons observés, a saturé rapidement. Cependant, la courbe de survie des larves n'a pas saturé, comme il le fut prédit. Bien que la prédiction que la courbe de survie devait initialement être positive soit supportée par les résultats, c'est plutôt une diminution de la survie qui fut observée avec l'augmentation du nombre

maximal de pucerons et, par conséquent, la diminution de la résistance. Deux expériences de laboratoire ont été réalisées afin d'isoler certains facteurs jugés importants dans l'explication du type de courbe de survie obtenue en champ. Dans la première expérience (Chapitre VI), le taux de rencontre entre des larves de coccinelles ainsi que le niveau de nourriture ont été contrôlés. Les résultats ont indiqué que les larves se cannibalisent entre elles d'une manière dépendante de la densité. La seconde expérience (Chapitre VII), réalisée avec des plants de sorgho encagés, des colonies de pucerons de différentes dimensions, et des introductions d'un nombre croissant de larves de coccinelles, a permis de reproduire les résultats obtenus en champ.

Les résultats présentés dans cette thèse suggèrent qu'une stratégie basée sur le déploiement de variétés avec une résistance intermédiaire stabiliserait l'interaction entre des ravageurs tels les pucerons, et des prédateurs telles les coccinelles.

## ABSTRACT

This thesis addresses the question of how plant resistance, by mediating herbivore number, affects the survivorship of the immature stages of a natural enemy. It was hypothesized that survivorship would initially increase and then saturate with decreased resistance and, thus, increased herbivore number. This hypothesis was tested in a maize agro-ecosystem composed of differentially resistant maize genotypes (DIMBOA-based resistance), the corn leaf aphid *Rhopalosiphum maidis* (Homoptera: Aphididae), and ladybird beetles (Coleoptera: Coccinellidae). Because plant resistance can be subject to variations mediated by the environment, the effect of plant stress on the survivorship of ladybird larvae was also investigated. Stress was imposed by either increasing plant density or by varying nitrogen supply.

A preliminary laboratory experiment indicated that DIMBOA did not influence the development of larvae of the ladybird *Propylea quatuordecimpunctata* fed aphids from a high DIMBOA genotype, but the combination of plant stress and resistance significantly increased pupal development time by about half a day (Chapter III). Mature ladybirds showed a significant preference for non-infested stressed greenhouse grown plants transferred to the field (Chapter IV). However, in the presence of aphids, the reproductive response of ladybirds in the field was related to peak aphid number, not to plant appearance *per se* when plant were nitrogen stressed (Chapter V).

In the greenhouse, a 2-fold increase in DIMBOA concentration was observed in stressed plants of a high DIMBOA genotype, whereas no significant variation was found between non-stressed and stressed plants of both intermediate and low

DIMBOA genotypes (Chapter II). The increase in DIMBOA concentration in stressed plants of the high DIMBOA genotype did not result in increased resistance to aphids in the laboratory because this genotype was already highly resistant. However, plant stress significantly decreased the growth of aphid colonies on the low DIMBOA genotype. In the field, variations in peak aphid number, with respect to maize genotype and to plant stress, were consistent with laboratory results (Chapter V). Thus, the deployment of intermediate or high DIMBOA genotypes can be considered to be stable strategies against aphids in this system.

In the field, the reproductive response of ladybirds saturated rapidly with respect to peak aphid number. However, the survivorship of immature ladybirds did not saturate, as predicted. Although survivorship initially increased with peak aphid number, as predicted, it decreased rapidly with further increase in peak aphid number. Two laboratory experiments were performed to isolate factors thought to be important in shaping the survivorship of ladybird larvae. The first experiment (Chapter VI), in which encounter rate among larvae and alternative food source were controlled for, indicated that cannibalism occurred readily and was density-dependent. In the second experiment (Chapter VII), small cages containing one sorghum (*Sorghum vulgare*) plant, aphid colonies of different size, and an increasing number of ladybird larvae were used. The pattern observed in the field was confirmed in this fully factorial experiment.

The results presented in this thesis suggest that an intermediate resistance strategy opens a window of stability in agro-ecosystems in which aphids are the consumers and ladybird beetles are the predators.

## ACKNOWLEDGMENTS

All and every one of us, be it as an observer of nature or as an experimenter, has a fondness for a particular system. Coming from a forestry background, I credit my supervisor, J.T. Arnason, for having convinced me to hop into fields I once looked at from the roadside. My gratitude to J.T. Arnason encompasses his communicating dynamism about chemical ecology and his wide interest for all things that make biology an unending journey. Also, I greatly acknowledge his support for providing resources and funding.

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TABLE OF CONTENTS

## LIST OF TABLES

**Figure 5.1**

Dynamics in the field of the tritrophic system composed of differentially resistant maize genotypes, the corn leaf aphid (*R. maidis*), and ladybird beetles. The inbreds CO273 (resistant), W64A (partially resistant), and bxbx (susceptible) were planted in 1994 and 1995. The inbreds A619Ht (resistant) and bxbx (susceptible) and the cross (F1s of A619Ht x bxbx; partially resistant) were planted in 1996. The percentage of plants with an exposed tassel (not shedding), the proportion of plants without an aphid colony, the number of aphids per plant (hollow circles) and the number of aphids per infested plant (filled circles), the number of ladybird eggs (1995 and 1996), and the number of ladybird pupae are shown in relation to Julian day. Each point represents the mean of 4 replicates with its standard error. A) 1994, low density (25 000 plants per ha or 42.5 cm between plants in a row). B) 1994, intermediate density (50 000 plants per ha or 20.0 cm between plants in a row). C) 1994, high density (100 000 plants per ha or 12.5 cm between plants in a row). D) 1995, low density. E) 1995, intermediate density. F) 1995, high density. G) 1996, high density (35 kg of N/ha). H) 1996, high density (70 kg/ha). ..... 98

**Figure 5.2**

Average daily temperatures recorded at the Central Experimental Farm, Agriculture Canada, Ottawa, during aphid infestations in 1994, 1995, and 1996. The horizontal dotted lines depict the optimum temperatures for

# — Chapter I —

## General Introduction: Ecological Windows for Stable Tritrophic Interactions

### 1.1 Control of insect pests by host-plant resistance and biological control in temporary agro-ecosystems

#### 1.1.1 Historical and theoretical considerations

The complete control of exotic pests by imported natural enemies (i.e. classical biological control) has historically been achieved mostly in agro-ecosystems greater than one year in duration (Hall and Ehler, 1979; Hall *et al.*, 1980; Stehr, 1982). Pest species able to successfully invade and reproduce in temporary habitats such as annual crops are characterized by high reproductive rates (*r*-selected). In itself, this attribute of these pest species is a formidable challenge for natural enemies. "Boom-and-bust" dynamics make it difficult for natural enemies to track these insect populations in space and time. Southwood and Comins (1976) captured the essence of this challenge in a synoptic model which includes a gradient of habitat duration. In their model, natural enemies can counteract and depress the rate of increase of herbivores when this rate is small (K-selected). This is, in itself, an attribute of species living in more persistent habitats. Thus, it seems that the challenge for practitioners of biological control is to open agro-ecosystems of short duration by acting on the rate of increase of the pest of interest.

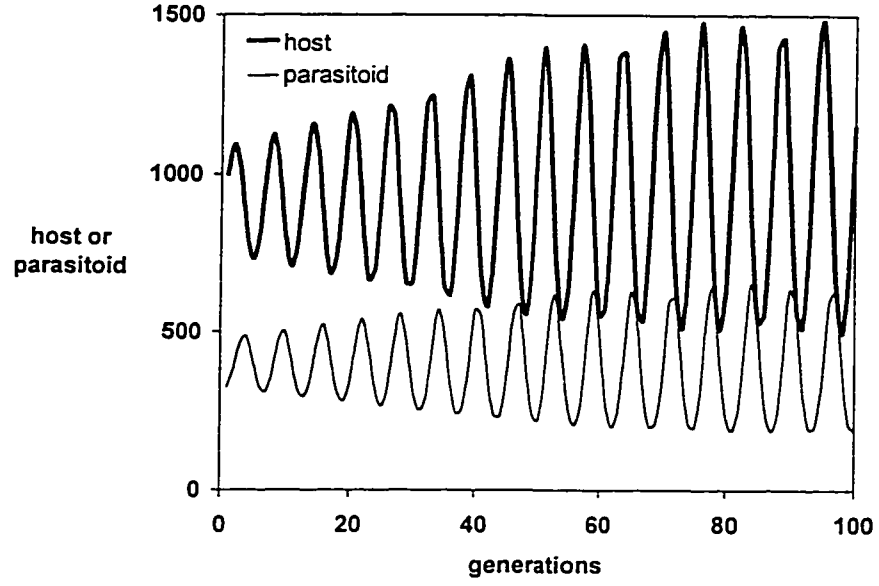
Acting on the rate of increase of agricultural pests in order to magnify the impact of natural enemies was first suggested by van Emden and Wearing (1965). Later, van Emden (1966), using Bombosch's (1963) simulation of an aphid-predator interaction, showed that aphid density is maintained constant more easily when the daily multiplication rate of the aphid is reduced. Qualitatively different mathematical models also generate lower equilibrium densities of insect herbivores in the presence of natural enemies as reproductive rates decrease (Beddington *et al.*, 1978).

Host-plant resistance would be a good mechanism for reducing the rate of increase of insect herbivores if control in agricultural habitats is to be achieved by biological means. As Lawton and McNeill (1979) summarized, herbivores in natural habitats are caught between "the devil and the deep blue sea", which are represented respectively by their natural enemies and by plants often nutritionally inadequate or toxic. Starks *et al.* (1972) were the first to experimentally demonstrate that a parasitoid wasp was able to maintain aphid abundance nearly constant for a period of over 4 weeks on a resistant variety of barley in the greenhouse. However, this very encouraging start was followed by numerous negative reports (*in* Boethel and Eikenbary [1986] and Hare [1992]). Antagonistic interactions between resistance factors and natural enemies at the individual level were found more often than not, leading to the impression that host-plant resistance and biological control are incompatible strategies. But, as Hare (1992), and Guttierez (1986) before

him, correctly pointed out, it is not possible to infer from observations at the individual level that antagonism will translate into incompatibility at the population level. In fact, the indirect effects of plant toxins on natural enemies may improve one important aspect of successful biological control, namely the stability of the interaction between the biological control agent and the pest.

To illustrate this, I will take data from McDougall *et al.* (1988) describing percent emergence of the solitary endoparasitoid *Diadegma terebrans* from European corn borer larvae fed meredic diets containing the relatively widely occurring alkaloid berberine, and I will use these data in simulations of a Nicholson-Bailey host-parasitoid model (Hastings, 1997). The simulations show that, compared to controls, the lower percent parasitoid emergence from hosts fed the highest concentration of berberine tested can stabilize the interaction (Fig. 1.1). Further reducing emergence rates, however, is conducive to the extinction of the parasitoid population, an unstable outcome indeed (*simulation not shown*). Hence, field experiments are the final frontier in which to test the compatibility of the two strategies, plant resistance and biological control.

Figure 1.1. Dynamics of a host-parasitoid interaction with differential mortality of the parasitoid mediated by the presence of a plant toxin (berberine) in the host diet (data from McDougall *et al.* [1988]). Top: divergent oscillations generated by percent parasitoid emergence from control hosts (80 %). Bottom: damped oscillations generated by percent parasitoid emergence from hosts exposed to berberine (61 %). Simulations from a Nicholson-Bailey model (Hastings, 1997).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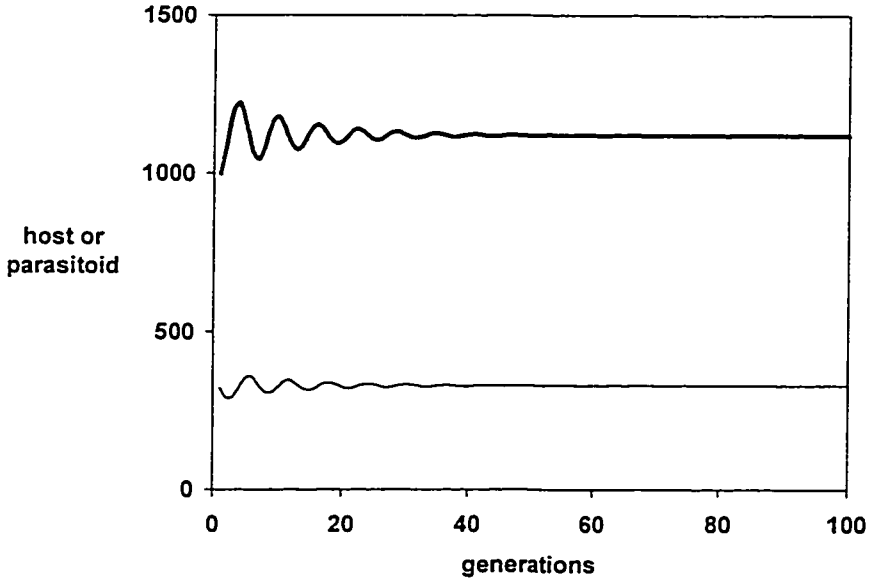
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### 1.1.2 Population dynamics in the field.

Since van Emden and Wearing's (1965) suggestion that decreasing the growth potential of pest species can enhance biological control, the interest of the scientific community has clearly shifted from questions relevant to population dynamics to questions pertaining to the details and mechanisms of direct and indirect effects of plants on natural enemies. This shift has improved our knowledge about how natural enemies use plant cues to locate their prey (Vet and Dicke, 1992) and how insect herbivores use plant chemicals to defend themselves (Rowell-Rahier and Pasteels, 1992) but, at the same time, it has hampered our capacity to generate hypotheses about tritrophic interactions in the field because of the many idiosyncrasies apparently involved. Here, I would like to swing the pendulum back by suggesting a way of looking at the interaction between natural enemies and insect pests as mediated by plant resistance in the field. I would like to do so by expanding beyond van Emden and Wearing's treatment of the question and by drawing on theories of predator-prey interactions, of food-chain dynamics, and of ecosystem stability.

I will begin with two assumptions. The first is that plants with very high levels of expressed resistance are not necessarily incompatible *per se* with biological control: the added mortality due to natural enemies is merely likely to be trivial compared to the level of protection already conferred by the resistance mechanism. Thus, at this end of the spectrum of plant resistance, the question of whether a

natural enemy is negatively (e.g. the insect pest is less sensitive than the natural enemy to the resistance factor) or positively (e.g. the natural enemy uses the resistance factor to locate its prey) affected by the resistance factor becomes irrelevant in terms of the level of control achieved. The second assumption concerns the other extreme of the resistance spectrum, with plants having very low levels of expression of a resistance factor. In this case, the dynamics of the pest species is likely to be driven by self-limitation most of the time as the population equilibrium is at, or near, carrying capacity. At this end of the spectrum, the impact of natural enemies is likely to be small if they are inefficient or, if they are efficient, stochastic events may allow the prey to escape control. The greater the rate of increase of the pest, the more it is likely to escape natural enemies in time. The second assumption, of course, derives from van Emden and Wearing's argument. These authors pointed out how plant resistance affects the rate of increase of insect pests, but they did not consider how resistance factors affect their carrying capacity.

The carrying capacity of a species is defined as the equilibrium density achieved under a given set of available resources and in the absence of predation (Begon *et al.*, 1986). As population density increases, intraspecific competition changes either or both the birth and the death rates until a point where they are equal and no change comes about in the growth of the population. At first glance, it may seem counter-intuitive to even consider competition to occur when plant

resistance results in low densities of insect pests. Indeed, the existence of competitive interactions among insect herbivores in natural habitats has been questioned on the basis of their usually low densities (e.g. Hairston *et al.*, 1960). Recent reviews have however gathered evidence that *interspecific* competition is detected at low population densities of insect herbivores (Damman, 1993; Denno *et al.*, 1995). The existence of *intraspecific* competition among insect herbivores has been debated (*see* Strong *et al.* [1984] *for a review concluding against the hypothesis, and* Dempster [1983] *for a review concluding in favor of the hypothesis*). However, if competition exists among different species which theoretically have greater niche differentiation, it is likely that it may be important between individuals of the same species exploiting the same resource as evidenced by a study in which *intraspecific* competition has been directly tested (Craig *et al.*, 1990)

Plant resistance can increase intraspecific competition at lower population densities in a number of ways. One general mechanism is that resistance decreases the edible portion of the plant (Lawton and McNeill, 1979). This is different from food quality reducing fecundity or increasing mortality independently of density, although both food quantity and quality intimately interact to reduce insect herbivore densities. An often cited example of this is the increased consumption rate observed in folivores to compensate for a decrease in the nutritional quality of plant containing digestibility-reducing compounds (Price *et al.*, 1980). Even in the absence of compensation, increased resistance, by prolonging development time,

can close a window of abundance of highly nutritious food. This is certainly true for most aphid species on their summer hosts throughout temperate areas, where a decrease in nutritional quality initiates a decline in reproductive rates before the density-dependent effect of crowding has reached its maximum (Dixon, 1985). Thus, resistance can create a relative shortage of food rather than an absolute one (White, 1978; Dempster, 1983). Whatever the mechanism, however, resistance can lead to a real or a technical decrease in the carrying capacity of the pest.

Hence, there are two points between which pest productivity can theoretically vary along a gradient of plant resistance. One is the hypothetical situation where the plant is completely immune from herbivore attack. In this case, the flow of energy does not extend beyond the plant as all the energy it captures is either locked in living tissue or goes into respiration. The other one is at the other extreme of the resistance spectrum, where the plant is completely susceptible to herbivore attack. In this case, the energy flows from the plant level to the herbivore level unimpeded, except for the energy contained in the cellulose and fiber portions of the plant (Abe and Higashi, 1991). The relevant question here is: As resistance decreases and more energy becomes available for the herbivore level, when can a third trophic level invade? A more important question from the perspective of control is: When, as prey productivity varies, can predators impact the herbivore population?

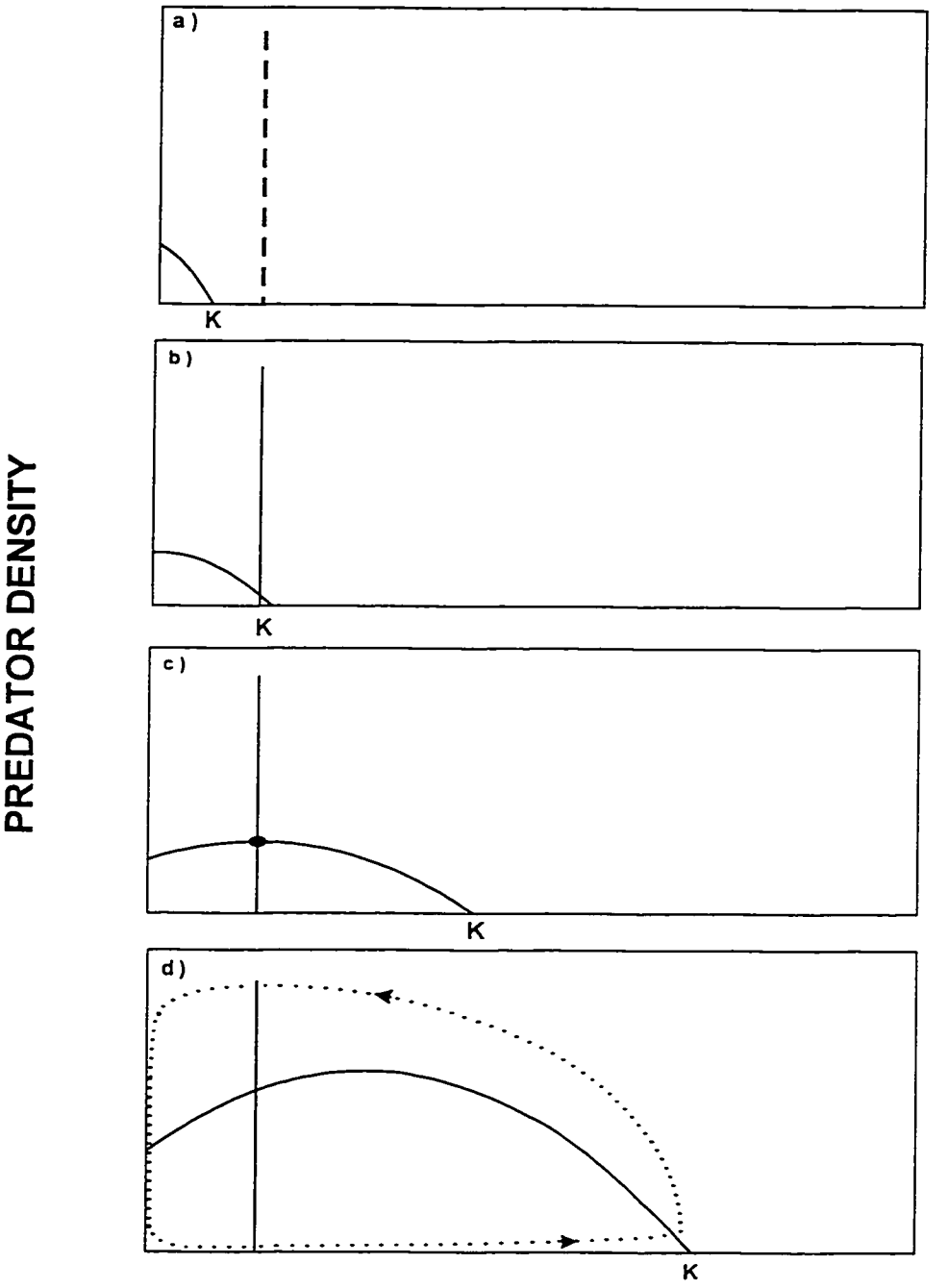
Similar questions have been addressed by ecologists interested in how plant productivity determines the length of food chains (Fretwell, 1977, 1987; Oksanen *et al.*, 1981). In their models, it is plant productivity that determines herbivore productivity and, in turn, the latter determines predator productivity, and so on for higher trophic levels until the diminishing flow of energy prevents the addition of an ultimate trophic level. The most interesting aspect of these model is that it allows ecosystems to grade from one integer food chain length to the next as plant productivity varies continuously. However, as this transition occurs, the relative importance of the top level in controlling the level below it also varies.

These views have been challenged on several grounds (*see* Power [1992] *for a discussion*), but mainly because the model treats whole ecosystems as food chains and whole trophic levels as a population of one species (Pimm and Lawton, 1978). Arguments have also been made that stability prevents the lengthening of food chains well before energy becomes a limiting factor (Pimm and Lawton, 1977; Abrams and Roth, 1994; Pimm and Kitching, 1987). I do not want here to choose sides, because as is often the case, reality probably lies between extreme views (Oksanen and Ericson, 1987). However, evidence has started to accumulate suggesting that, in terrestrial systems, food webs behave like food chains and that the removal of a top predator has effects on trophic levels below (Rosenheim *et al.* 1993; Gómez and Zamora, 1994; Marquis, 1994; Dial and Roughgarden, 1995; Moran *et al.*, 1996).

The fact that it can be experimentally demonstrated that natural enemies can indirectly be beneficial to the plant level should, however, come as no surprise. Hunter and Price (1992) suggested that the most important question is not to ask if things are controlled from the top, but rather under which circumstances things are controlled from the top. This is certainly a first step when systems are looked at as a whole, but it can be further argued that the impact of natural enemies is not the only crucial aspect in tritrophic systems. Although a low pest equilibrium density is a logical goal in agro-ecosystems, it has been recognized that maximizing the impact of natural enemies on insect herbivores is often achieved at the sacrifice of stability, and vice-versa (Beddington *et al.*, 1976; Beddington *et al.*, 1978; Murdoch, 1990). In the context of combining plant resistance and biological control, the relevant question becomes: can both stability and high enemy impact be achieved in a given system?

I have used phase-plane analysis of predator and prey isoclines generated by a Lotka-Volterra model (*as in* Lundberg and Fryxell, [1995]; Appendix I) to provide a theoretical answer to the question (Fig. 1.2). The model is suited for true predators in which several prey are needed to complete a cycle and hence is not appropriate for parasitoids although the general outcome of the model may apply to these as well (Hastings, 1997). For the sake of generality, the model includes density-dependence in the prey population and the predators exhibit a saturating functional response.

Figure 1.2. Phase-plane analyses of predator and prey isoclines with increasing carrying capacities (K) of the prey population as determined by host-plant resistance (*see text*). The rate of increase of the prey is maintained constant in all four simulations. The isoclines are derived from Lotka-Volterra equations (*see* Appendix I) which include density-dependence in the prey population (self-limitation) and a saturating functional response on the part of the predator (*as in* Lundberg and Fryxell, [1995]). The vector (*dotted line*) in d) represents the trajectory of the coupled predator-prey densities. All plots are on the same scale.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K
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PREDATOR DENSITY
HERBIVORE DENSITY

In Lotka-Volterra models, the efficiency of a predator is a constant as is defined by the minimum biomass of prey needed to produce positive growth in the predator population (Rosenzweig and MacArthur, 1963). Thus, the position of the vertical predator isocline is not affected by changes in the carrying capacity of the prey (Rosenzweig, 1971). Maly (1967) has reported that he obtained experimental isoclines which conform to the general form of the isoclines predicted by Lotka-Volterra models with prey self-limitation and a predator saturating functional response. The interplay of self-limitation in a prey population (*Paramecium aurelia*) and the efficiency of a predator (*Didinium nasutum*) has been examined in the laboratory by Luckinbill (1973). Reducing the resources to the prey increased self-limitation (decreased its carrying capacity) and stabilized the interaction. Increasing the viscosity of the medium reduced the efficiency of the predator (thus, moving its isocline to the right) and this, also, stabilized the interaction. Hence, both parameters, the carrying capacity of the prey and the efficiency of the predator, on which hinge the stability properties of the model presented in figure 1.2, have been shown to be important in the laboratory.

In the simulations presented in figure 1.2, the carrying capacity of the prey has been set at four different sizes to simulate the effect of plant resistance on population densities of the prey. The outcomes of the model show that: 1) very high levels of plant resistance prevent the addition of a third trophic level in the system (Fig. 1.2a), 2) a slight decrease in resistance allows more prey to be present

but barely enough to maintain a predator population (Fig. 1.2b), 3) intermediate resistance allows prey productivity to sustain a viable predator population (Fig. 1.2c), 4) low resistance increases prey productivity but generates cycles of years with endemic populations followed by years of outbreaks (Fig. 1.2d). In this latter case, the trajectories come so close to the axis that there is a high probability of extinction, a phenomenon called by Rosenzweig (1971) the "paradox of enrichment".

In terms of stability, which has been defined in a number of ways (*see* van Emden and Williams [1974]) and will be taken here as the restricted fluctuation of prey number over time (Murdoch, 1994), the last simulation (Fig 1.2d) in which plants express low levels of resistance, is clearly the most unstable situation. Yet, this simulation illustrates that a predator can have a great impact on its prey. In contrast, the first two simulations (Fig. 1.2a-b) indicate that natural enemies will have no or little impact on the herbivores with plants expressing high resistance levels. Yet, these systems are stable. Only when plants express intermediate levels of resistance that both stability and high enemy impact can be achieved simultaneously.

It should be emphasized that if the model of Lundberg and Fryxell (1995) points to the conclusion that relatively high resistance levels are stable outcomes dynamically speaking, considerations should be given to the possibility that

herbivores evolve counter-adaptations. Hence, if high resistance results in a low pest equilibrium density, this gain should be weighed in relation to the number of generations a given pest species will need to overcome the resistance mechanism deployed. This number of generations can be modeled and predicted from selection experiments (Gould, 1986ab, 1988; Ferro, 1993; Tabashnik, 1994). The lower the number of generations needed for the resistance mechanism to break down, the less stable this strategy would be compared to the intermediate resistance strategy. In the light of recent assessments of the number of generations needed for resistance to breakdown (Ferro, 1993; Tabashnik, 1994), which indicate that as little as four generations are sufficient, the intermediate resistance strategy would seem to be the most appropriate in terms of stability (Fig 1.2c).

A more problematic situation arises when prey productivity minimally meets the requirement for a predator population to exhibit positive growth (*e.g.* Fig. 1.2b). In this situation, predators are likely to feed on weakened prey items as the dynamics in the prey population is driven by intraspecific competition. These may be individuals wandering about for more nutritious and less toxic plant tissues, increasing their exposure to predators. If there is a genetic correlation between the mobility of these individuals and the risk of predation, predators in this scenario are likely to hasten the rate at which herbivores overcome resistance (Gould *et al.*, 1991). This also can be modeled and the number of generations needed for resistance to break down predicted.

## 1.2 Testing the intermediate resistance hypothesis

The outcome of the simulation with intermediate resistance level (*i.e.* intermediate K; Fig. 1.2c) is a stable point at the intercept of the predator and the prey isoclines. This means that densities of both the predator and the prey will tend to rapidly return to this point following small perturbations, which are frequent in temporary agro-ecosystems. One way of determining the acceptability of the intermediate resistance strategy from an agricultural point of view is to know whether the resistance level which produces the sort of interaction shown in figure 1.2c sets the carrying capacity of the prey at, or near, the economic threshold level. Natural enemies in this scenario thus act as an insurance policy preventing insect pests from reaching the economic threshold level with the likely accrued advantage that a high selection pressure is not imposed on the pest population as it may arise using a high resistance level strategy alone.

Testing the intermediate resistance hypothesis in the field would ideally require monitoring population densities of both the predator and the prey over several generations. The appropriate experiment would also have to include several plant genotypes differentially resistant to the insect pest. Furthermore, each genotype included in the experiment would have to be at the base of an independent tritrophic system, an unlikely possibility in the field. However, the model used here indicates that the general outcome of a saturating functional

response on the part of the predator is that the rate of change of the predator population is also a saturating function of prey density.

A mathematical treatment of the variation in the rate of change in the predator population with respect to prey density is given in Appendix II. But, it can be verbally stated that there are two prey densities between which the rate of change in the predator population varies (Tanner, 1975). The first prey density is the minimal density of prey required for the predator to survive. Hence, until this minimal prey density is reached, there is no growth in the predator population. The second prey density is the one at which the predator cannot consume more prey per unit of time because pursuing, subduing, and consuming a prey take some time (Holling, 1959). Hence, at this second prey density, the rate of change in the predator population is maximal. Between the two critical prey densities, the rate of change in the predator population increases rapidly initially because handling a prey item once found is not limiting, but as prey density increases handling time becomes more and more limiting. Hence, the rate of change in the predator population is a decelerating, or saturating, function of prey density.

Since a portion of the energy acquired by the predators through prey consumption is devoted to the production of new predators, it follows that the survivorship of the immature stages of a predator would also be a decelerating, or saturating, function of prey density. Hence, monitoring the survivorship of

immature predators in relation to prey density, as mediated by plant resistance in the field, is the variable I have chosen to test the intermediate resistance hypothesis. The range of prey density over which the survivorship of immature predators would start to saturate would be the window mediated by plant resistance which stabilizes the interaction between the predator and its prey. The null hypothesis against which this hypothesis was tested is that the survivorship of immature predators is independent of prey density. In other words, the null hypothesis is that plant resistance level, as it bears on prey productivity, does not contribute to the stability of the system.

Clearly, the approach I have taken so far to describe the interactions in a tritrophic system can be decomposed in bottom-up and top-down forces acting in conjunction, not in isolation (Hunter and Price, 1992). At the plant level, resistance mediates prey productivity. In turn, the life history parameters of the predator, the third trophic level, determine how much of prey productivity will transfer into predator biomass. More importantly, the model used (Fig. 1.2) predicts that there is a narrow window of prey density which stabilizes the flow of energy between trophic levels. It follows that if the resistance factor at the plant level is sensitive to extrinsic factors such as abiotic stresses (DiCosmo and Towers, 1984; Gershenzon, 1984; Herms and Mattson, 1992), the whole system becomes unstable. Hence, I have examined the effect of plant stress on prey productivity and the consequences for the survivorship of immature predators.

## 1.3 The system

I conducted my experiments in a maize (*Zea mays* L.) agro-ecosystem consisting of the corn leaf aphid *Rhopalosiphum maïdis* (Fitch) (Homoptera: Aphididae) and predatory ladybird beetles (Coleoptera: Coccinellidae). Maize contains the toxic secondary metabolite DIMBOA, an hydroxamic acid, which acts as a resistance factor against several insect pests, including aphids (Niemeyer, 1988). I selected differentially resistant maize genotypes on the basis of previously reported DIMBOA content and field trials (Long *et al.*, 1977; Beck *et al.*, 1983), and of preliminary experiments. Figure 1.3 shows an aphid colony on the high DIMBOA genotype A619 after the plant has been naturally infested and caged for about one month. Figure 1.4 shows an aphid colony on the DIMBOA-deficient genotype bxbx, also one month after being caged.

The corn leaf aphid *R. maïdis* is a cosmopolitan species that feeds on several wild and cultivated Poaceae and which are completely parthenogenetic in North-America (Wildermuth and Walter, 1932). *R. maïdis* does not seem to overwinter north of Texas (Steiner *et al.*, 1985; Voegtlin *et al.*, 1987). During spring, air currents transport winged morphs to northern sites (Cartier, 1957). In Ontario and Québec, *R. maïdis* can be collected in traps or seen on wheat and barley as early as April, but colonies on maize are usually not found before June (Foott, 1977; Coderre, 1983).

Figure 1.3. A colony of *Rhopalosiphum maidis* on the high DIMBOA genotype A619 one month after infestation. The plant was caged.

Figure 1.4. A colony of *Rhopalosiphum maidis* on the DIMBOA-deficient genotype bxbx one month after infestation. The plant was caged.

The phenology of *R. maïdis* infestations on maize is characterized by two peaks of different importance in terms of number of individuals (Foott and Timmins, 1973; Coderre, 1983). The first and more modest peak corresponds to late whorl and tassel infestations. The whorl provide protection to the aphids because it completely encloses the tassel. The subsequent decrease maybe in part due to predation because the colonies become completely exposed at anthesis, but mutual interference or lower nutritional quality, which induce the production of winged morphs, may also contribute to the decline (Dixon, 1985). The initial infestation stage lasts about one month (Wright and Laing, 1980). Migration then spreads infestation to more plants than were initially attacked and the development of these colonies is responsible for the second and more important peak of the season. The silks and the husk are colonized during the second stage of the infestation, which can last until harvest.

It is generally believed that only heavy aphid infestations can reduce yield in maize. However, Everly (1960) observed that even light infestations on the tassel can cause up to 10% loss in yield. It has been suggested that reduced yield results directly from the diversion of assimilates as a consequence of aphid feeding rather than honeydew production interfering with pollination (Neiswander and Triplehorn, 1961; Everly *et al.*, 1965; Foott and Timmins, 1973). Aphid feeding can have a significant impact on the carbon balance of the plant since for any amount of nitrogen diverted, the plant has to assimilate a much greater amount of CO2 to

replace this loss (Raven, 1983). It is not known if honeydew can interfere with CO2 uptake by clogging the stomata, but when abundant, it acts as a very strong desiccant by drawing out water osmotically through the leaf epidermis (*in* Comeau, 1992). Honeydew also encourages fungal growth, reducing the photosynthetic capacity of the plant (Risebrow and Dixon, 1987). Kiniry *et al.* (1992) have shown in a recent study that grain filling depends mostly on photosynthesis after anthesis. Thus, honeydew production and assimilate diversion might have additive or synergetic effects on yield. By interfering with the plant capacity to acquire resources, aphids may increase effects of plant stress. This was seen most dramatically when severe infestations by *R. maïdis* (many hundreds on most of the tassel) coincided with low rainfall during the tasseling stage, reducing yield up to 91,8 % whereas losses did not exceed 58,9 % in well-watered plants (Foott and Timmins, 1973).

The natural enemy complex of *R. maïdis* in maize fields of southern Québec was studied over five years by Coderre (1983). He found that ladybird beetles (Coleoptera: Coccinellidae) largely dominated in terms of abundance and diversity. Common lacewings (Neuroptera: Chrysopidae), brown lacewings (Neuroptera: Hemerobiidae), and hoverflies (Diptera: Syrphidae) were present sporadically or in very low numbers. These are the predators generally associated with aphids on cereals (Dixon, 1977). Parasitoïds or mummies were not reported by Coderre (1983), although some *Aphelinidae* spp. (Hymoneptera) are known to

attack *R. maïdis* in the United States (Jackson *et al.*, 1970; Archer *et al.*, 1974; Feng *et al.*, 1992).

Observations of the abundance and diversity of ladybird beetles in maize fields of southern Québec and Ontario yielded different results (Smith, 1971; Foott, 1973; Wright and Laing, 1980; Coderre, 1983). Dominant species reported in one study were not found in others, or were only represented by few individuals. The total abundance of ladybird beetles varied from year to year, but was not always proportional to aphid abundance. Their rate of arrival in maize fields and their numerical increase followed the same pattern consistently: few adults arrive before anthesis and subsequent increases seem to be the result of reproduction within the field rather than immigration. Foott (1973) considered that the numerical response of ladybird beetles was too weak to achieve successful control of aphids in maize fields. Moreover, by the time ladybird beetles become numerically important, damage caused by aphids has already occurred (Foott and Timmins, 1973).

Two phenomena complicate the task of ladybird beetles in maize fields. First, aphids have partial refuges in the late whorl and in the husk. Coderre and Tourneur (1986) found that up to 84% of the aphid population hid in the husk during ear maturation. Second, when pollination is over and aphids start colonizing the ears, there is an increase in the number of plants infested (Coderre, 1983). Thus, when the aphids infest the ears, there is a high overall number of

aphids in the field, but they are scattered in many low density and partially hidden colonies.

Although previous reports indicate that *R. maidis* is not a good candidate for control by biological agents such as ladybird beetles, the maize agro-ecosystem possesses attributes that facilitate testing of the intermediate resistance hypothesis. First, the phenology of *R. maidis* infestation is characterized by well-defined temporal boundaries, i.e. migration initiates infestation and the emergence of the tassel from the whorl induces the decline in the colonies. Second, the refuge conferred by the whorl allows the direct measurement of the effect of different resistant levels on the maximum, or peak, number of aphids. In turn, this peak number sets the limit to the number of prey available for the immature ladybirds to forage on.

### 1.4 Structure of the thesis

This thesis is divided into 8 chapters, including the present one. In Chapter II, I examine the effect of plant stress on the stability of the resistance factor, DIMBOA, in the greenhouse: the growth of aphid colonies on stressed and non-stressed plants of differentially resistant maize genotypes and DIMBOA concentrations on these plants are measured. In Chapter III, I investigate the

possibility that the survivorship and the development of immature ladybird beetles are affected by feeding on aphids from a resistant maize genotype or from stressed plants, an indirect plant effect. In Chapter IV, I look at another possible indirect plant effect, this time on the behavior of adult ladybird beetles. In the latter chapter, I am asking if ladybird beetles demonstrate a preference for a particular maize genotype, or for stressed plants as opposed to non-stressed plants. The results presented in these chapters would help interpret the patterns observed in the field experiments, which are the focus of Chapter V and which were implemented to test the intermediate resistance hypothesis. In Chapters VI and VII, I report the results of laboratory experiments conducted to further understand patterns observed in the field.

Each of the experimental chapters stands alone as a specific question is asked. It is in the last chapter (VIII) that I discuss more generally the results presented in this thesis. In particular, I discuss the possibility that natural enemies are selective agents for the level of plant defenses.

# — Chapter II —

## PLANT STRESS AND DIMBOA-BASED MAIZE RESISTANCE TO THE CORN LEAF APHID, *RHOPALOSIPHUM MAIDIS* FITCH..

### 2.1 Introduction

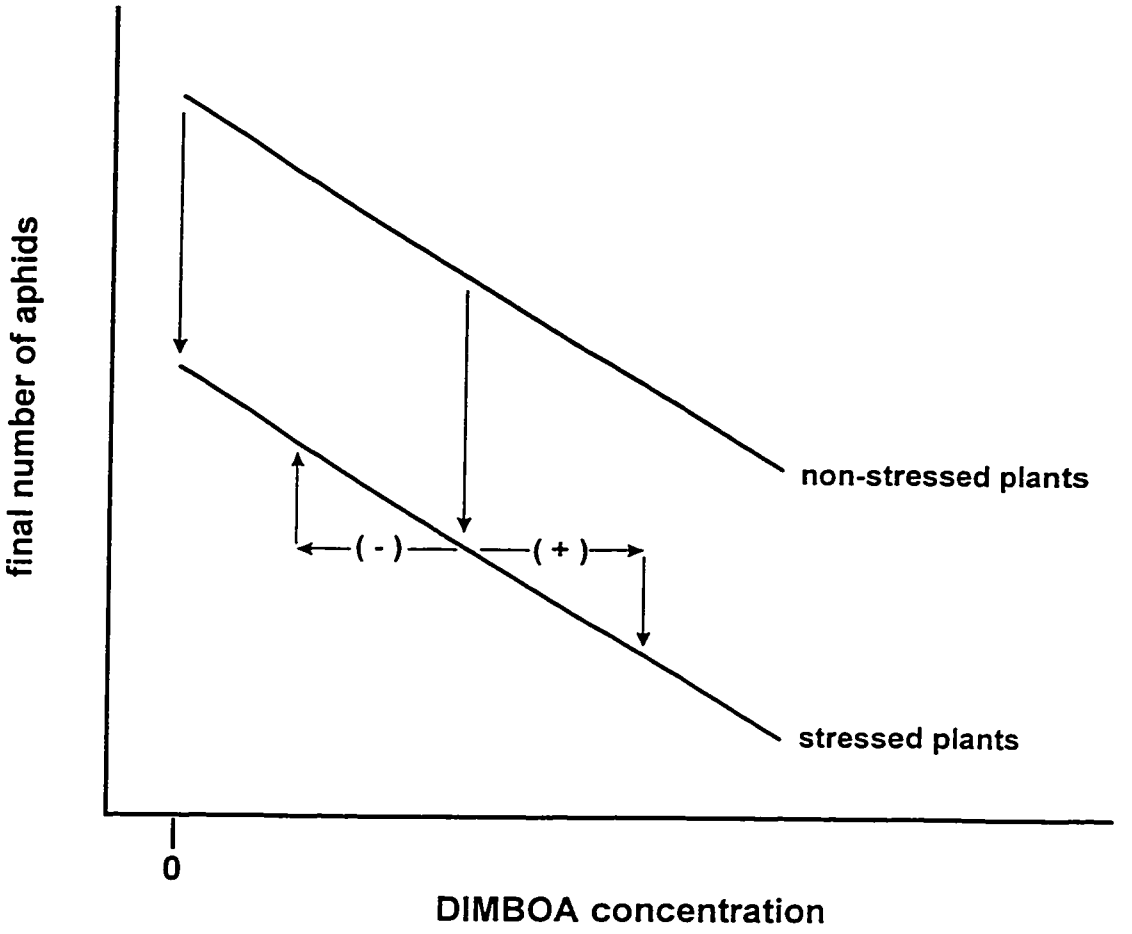
The stability of crop resistance to insect pests under varying abiotic conditions is as essential as the stability of yield and other agronomic traits if resistance is to be implemented as a control strategy. Yet, stability of resistance traits is rarely investigated *per se* (*but see*, McMurtry [1962], Faris *et al.* [1979], and Sharma and Lopez [1991]). Traditionally, a large number of genotypes is screened for low damage score under natural or artificial infestation trials and, once resistant germplasms are found, their genes are incorporated into lines possessing desirable agronomic traits and further selection cycles concentrate on the latter (Kogan, 1982). However, resistance factors derived from the secondary metabolism of plants are known to be sensitive to the conditions under which the plants are growing (Painter, 1954; DiCosmo and Towers, 1984; Gershenzon, 1984; Herms and Mattson, 1992) and this will influence operational resistance in the field.

The concentration of DIMBOA, an hydroxamic acid found in plants of the Poaceae family and believed to be a resistance factor against several insect species

(Niemeyer, 1988), has been found to vary depending on growing conditions. In maize seedlings, DIMBOA concentrations were increased by iron deficiency (Manuwoto and Scriber, 1985a) and water stress (Richardson and Bacon, 1993), whereas concentrations remained unchanged or decreased with nitrogen deficiency (Manuwoto and Scriber, 1985a,b).

The effect of plant stress on DIMBOA concentration in specific parts of maize plants infested by the corn leaf aphid, *Rhopalosiphum maidis*, the late-whorl and tassel tissues, has never been investigated. Furthermore, the response of this aphid species to the combined influence of plant stress and DIMBOA concentration is unknown. The corn leaf aphid feeds on young expanding plant tissues (Foott and Timmins, 1973; Coderre, 1983). Because it feeds on tissues in which translocation of assimilates occurs at high rate, I hypothesized that plant stress will have a negative impact on the rate of increase of this species, even without considering the effect of DIMBOA. However, predictions about the outcome of plant stress and DIMBOA concentration on the corn leaf aphid are impeded by the fact that DIMBOA may increase or decrease with plant stress. Coupled with the expected negative effect of plant stress on the aphid, a decrease in DIMBOA concentration may free the aphid from the adverse effects of the secondary metabolite and confound the impact of plant stress. On the other hand, an increase in DIMBOA concentration may magnify the effect of plant stress on the aphid. Figure 2.1 illustrates these outcomes.

Figure 2.1. Predicted outcome of plant stress and DIMBOA concentration on aphid number. Plant stress alone ([DIMBOA] = 0) is predicted to have a negative effect on the corn leaf aphid. DIMBOA concentration may decrease (-) or increase (+) with plant stress.


final number of aphids
non-stressed plants
( - )
( + )
stressed plants
0
DIMBOA concentration

The model proposed here is an additive one since it assumes a constant negative effect of plant stress on the aphid, whatever the DIMBOA concentration is. According to this model, the existence of a significant statistical interaction between plant stress and maize genotypes differentially resistant to the corn leaf aphid should not be interpreted as a biological interaction with DIMBOA. The variation in DIMBOA concentration between stressed and non-stressed plants of different genotypes must be known before reaching such a conclusion. Therefore, the present investigation was undertaken using stressed and non-stressed plants of DIMBOA-deficient and high-DIMBOA maize inbreds along with the cross between these parental lines for which DIMBOA concentrations were determined and on which the growth of corn leaf aphid colonies were monitored.

## 2.2 Material and methods

### 2.2.1 Plant material

The seeds of the high-DIMBOA inbred (A619Ht) were obtained from Dr. R. I. Hamilton (Eastern Cereals and Oil Seed Research Centre, Central Experimental Farm, Agriculture Canada, Ottawa) and Dr. P. A. Peterson (Department of Agronomy, Iowa State University, Ames, USA) provided the seeds of the DIMBOA-deficient inbred (bxbx; original source: Dr. L. Gracen, Cornell University,

Ithaca, USA). In 1994, I proceeded to make the cross between these two inbreds and used the F1 seeds in the present study to generate plants with intermediate levels of DIMBOA.

Plants of the three maize genotypes were grown during the summer of 1996 in the greenhouse in 12 L pots filled with a maize soil medium commonly used at the Central Experimental Farm (Agriculture Canada, Ottawa). Water was provided through an automatic watering system and fertilizer was applied once a week (500 ml/pot of a solution made of 25 g of 20-20-20 N-P-K diluted in 1 L of tap water). For the stress treatment, three plants were grown in the same pot, whereas non-stressed plants were grown singly. Imposing stress in this manner was preferred over controlling for the amount of nutrients because of the high demand of maize plants for water. Under warm temperatures, potted maize plants in the greenhouse need to be watered at least twice daily. This results in considerable and unavoidable leaching of nutrients. Hence, growing more than one plant per pot was thought to provide a more uniform level of stress as well as a comparable situation to field conditions were plants are grown at different population densities. Although plants in the stress treatment competed for light, water, and nutrients, the latter factor was probably the most limiting since none of the plants were allowed to wilt and plants were only *ca.* 5 cm closer to each other in the stress treatment than when planted singly.

To obtain plants of the same physiological age across treatments, F1 plants were started 5 days later than parent plants, and non-stressed plants 3 days later than stressed plants. In addition to this precaution, only plants with a non-exposed, but well developed, tassel were selected for both the chemical analyses and the aphid trial.

### 2.2.2 Extraction and determination of DIMBOA

Whorl leaves and tassel samples were taken from four different plants from each combination of maize genotype and stress level. The yellow sections (basal) of the two most inner leaves of the whorl and the tassel were collected and stored at -20 °C. Before freeze-drying the samples, they were thawed for 30 minutes to allow the enzymatic hydrolysis of DIMBOA-glucoside (Massardo *et al.*, 1994). Dried samples were reduced to powder using a Willey Mill© and passed through a 40 size mesh before storage in a freezer until extraction.

The extraction procedure was similar to that of Bervingson (1994). For each sample, 0.50 g of powder was extracted, except when the amount of sample was insufficient if the extraction had to be repeated, in which case 0.25 g was used. Details of the extraction procedure and of the solvent system used with the HPLC are provided in Appendix III. Recovery of DIMBOA using this procedure was 92.3 %.

### 2.2.3 Aphid trial

The corn leaf aphids for this study were from a clonal colony established on sorghum (*Sorghum vulgare;* Cargill hybrid, common soudangrass). Apterous morphs in their penultimate (L3) or ultimate (L4) nymphal instars were selected to initiate colonies on the plants from the different combinations of genotype and stress level. The rationale for using these instars is as follows. Preliminary results showed that when first instars were used, most of them never settled on even mildly resistant maize. Thus, such high initial mortality rates would lead to an overestimation of the effect of DIMBOA concentration and possibly to a low resolution between intermediate and high-DIMBOA genotypes. The use of apterous adults was also prevented because they may have already given birth to some nymphs before being switched to the experimental plants. Hence, the use of L3-L4 nymphs was a compromise between the two other alternatives.

Greenhouse grown plants similar to those selected for the chemical analyses were brought to an environmental chamber (25 °C, 70 % r.h., and 16L:8D) and inoculated with five *R. maidis* nymphs per plant, three plants per treatment. The whorl of the inoculated plants were covered with a sleeve cage and the number of aphids alive was counted after a period of 8 days.

### 2.2.4 Statistical analyses

DIMBOA concentrations were statistically analyzed by ANOVA as a fully factorial, completely randomized design. To test the model presented in figure 2.1, independent regression analyses were performed for each stress level, with the mean final number of aphids as the dependent variable and the mean concentration of DIMBOA as the independent variable. The slopes of each regression lines were then compared with a Student's *t*-test for statistical differences (Zar, 1974). Rejection of the null hypothesis of identical slopes would invalidate the model in which the effect of DIMBOA is additive to the effect of plant stress.

## 2.3 Results

In late-whorl and tassel tissues of non-stressed maize, the concentration of DIMBOA varied by over one order of magnitude between the DIMBOA-deficient parent, bxbx, and the high-DIMBOA parent, A619Ht, while the concentration was intermediate in the plants from the cross (Table 2.1). The effect of plant stress on the concentration of DIMBOA depended on genotype, as indicated by the significant interaction term from the ANOVA (Table 2.2). DIMBOA concentration increased more than 2-fold in stressed plants of the high-DIMBOA parent, A619Ht (d.f. = 6, $t$ = -2.685, $p$ = 0.0363; Table 2.1), whereas no significant effect of plant

Table 2.1. Concentration of DIMBOA (mg/g d.w.) in non-stressed and stressed plants of the DIMBOA-deficient maize bxbx, the high-DIMBOA maize A619Ht, and the cross (F1). Sample size is 4 for each treatment.

| | Genotype | | |
|---|---|---|---|
| | bxbx | F1 | A619Ht |
| non-stressed | 0.04 (0.02)[†] | 0.08 (0.02) | 0.17 (0.06) |
| stressed | 0.03 (0.03) | 0.10 (0.02) | 0.39 (0.06) |

[†]mean ± one standard error

Table 2.2 ANOVA on DIMBOA concentration.

| Source | *d.f.* | *M.S.* | *F-value* | *P* |
|---|---|---|---|---|
| genotype | 2 | 0.134 | 21.190 | <0.0001 |
| stress | 1 | 0.036 | 5.688 | 0.0283 |
| interaction | 2 | 0.033 | 5.224 | 0.0163 |
| error | 18 | 0.006 | | |

stress was found for the DIMBOA-deficient parent, bxbx (d.f. = 6, $t$ = 0.187, $p$ = 0.8576; Table 2.1), or for the cross (d.f. = 6, $t$ = -0.500, $p$ = 0.6346; Table 2.1).

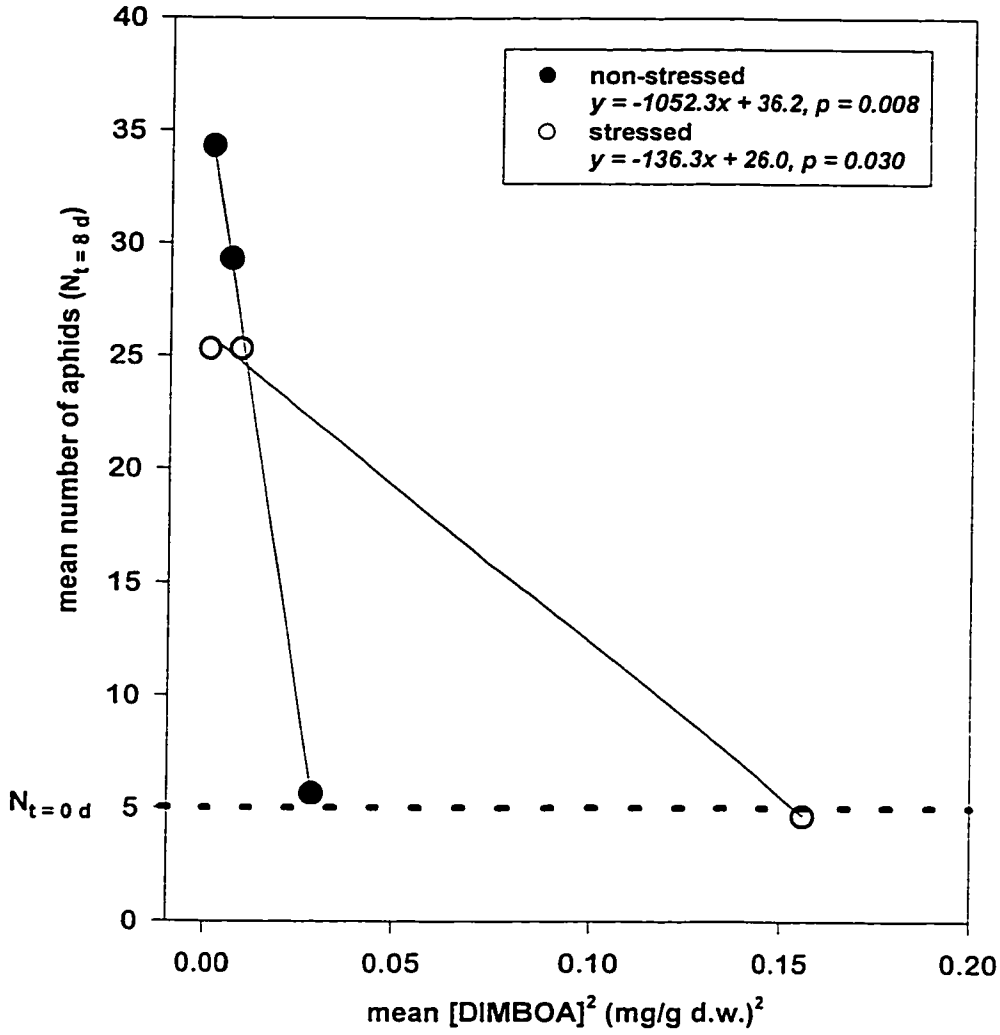
The means of the number of aphids 8 days after inoculation on the plants from the different combinations of genotype and plant stress are presented in Table 2.3. These means were used to compute independent regression equations relating final aphid numbers to mean DIMBOA concentrations for each stress level. Mean DIMBOA concentrations were squared to linearize the data in order to meet assumptions of regression analysis, though the biological interpretation underlying this non-linear relationship between the growth of aphid colonies and DIMBOA concentration has been related to the increasingly deterrent property of the compound to aphids as concentration increases (Argandoña *et al.*, 1983; Corcuera *et al.*, 1985; Niemeyer *et al.*, 1989; Givovich and Niemeyer, 1991; Givovich *et al.*, 1992; Nicol *et al.*, 1992). The mean final number of aphids was significantly and negatively related to the squared mean DIMBOA concentrations for both the non-stressed and the stressed plants (Fig. 2.2). However, the decrease in the mean final number of aphids as the squared mean DIMBOA concentrations increased was significantly steeper for the non-stressed plants than for the stressed plants (d.f. = 2, $t$ = -30.82, $p$ = 0.0011). This result invalidates the model in which the effect of DIMBOA is additive to the effect of plant stress, though the predicted decrease in final aphid number due to plant stress and in the absence of DIMBOA is supported by the data, as indicated by the greater intercept estimate for the non-stressed plants regression (Fig. 2.2).

Table 2.3 Final number of aphids on non-stressed and stressed plants of the DIMBOA-deficient maize bxbx, the high-DIMBOA maize A619Ht, and the cross (F1), 8 days after inoculation with 5 aphids (L3-L4) per plant. Sample size is 3 for each treatment.

| | Genotype | | |
|---|---|---|---|
| | bxbx | F1 | A619Ht |
| non-stressed | 34.3 (3.8)[†] | 29.3 (3.7) | 5.7 (2.3) |
| stressed | 25.3 (1.2) | 25.3 (0.9) | 4.7 (3.7) |

[†]mean ± one standard error

Figure 2.2 Interaction between plant stress and DIMBOA (mean concentrations squared to linearize) on final aphid number. Colonies were initiated with 5 aphids ($N_{t=0\,d}$). The slopes are significantly different (d.f. = 2, $t$ = -30.82, $p$ = 0.0011).


non-stressed
y = -1052.3x + 36.2, p = 0.008
stressed
y = -136.3x + 26.0, p = 0.030
mean number of aphids (N t = 8 d)
N t = 0 d
mean [DIMBOA]2 (mg/g d.w.)2
0
5
10
15
20
25
30
35
40
0.00
0.05
0.10
0.15
0.20

## 2.4 Discussion

The effect of plant stress on DIMBOA concentration in late-whorl and tassel tissues of maize depended on genotype. Only the high-DIMBOA inbred, A619Ht, showed a significant variation of the concentration of the secondary metabolite when stressed. The more than 2-fold observed increase in the mean concentration of DIMBOA in the stressed plants of this high-DIMBOA inbred did not, however, translate into increased resistance to the corn leaf aphid compared to the non-stressed plants. The aphid colonies did not develop on either the non-stressed or the stressed plants of this inbred (Fig. 2.2).

Using a different analytical method, Beck *et al.* (1983) found the concentration of total hydroxamic acids in the inbred A619 to be 0.059 mg/g of fresh weight (i.e., 0.20 mg/g d.w., assuming 70 % water), which is similar to the result obtained here for the single hydroxamic acid DIMBOA in the non-stressed plants (0.17 mg/g d.w.). These authors also found that colonies of the corn leaf aphid were unable to grow on this inbred in their greenhouse trial. Thus, it would appear that resistance level to the corn leaf aphid remains stable, despite the variation in the concentration of DIMBOA due to plant stress reported here for the high-DIMBOA inbred A619Ht[1]. Other types of stress along with a wider range of

[1] The difference between inbred A619Ht and A619 is that the genes for resistance to the pathogen *Helminthosporium triticum* have been incorporated in the former.

stress levels would have to be tested to confirm this conclusion since it has been shown that DIMBOA concentration may increase or decrease depending on the type of stress imposed (Manuwoto and Scriber, 1985a,b; Richardson and Bacon, 1993).

Although it was found that plant stress affects negatively the corn leaf aphid, as hypothesized, the effect of DIMBOA was not additive, as suggested by the model shown in figure 2.1. One alternative hypothesis, a synergetic effect between DIMBOA and plant stress, is also not supported by the data presented here (Fig. 2.2). The data rather point to a second alternative in which plant stress results in lower ingestion rates of DIMBOA. Aphids on herbaceous plants have to feed actively because of the low sap pressure (Raven, 1983). Wearing and van Emden (1967) have discussed the importance of sap pressure for aphids, especially for species feeding on the apex of plants. Sumner *et al.* (1986), using hydroponically grown winter wheat with different amounts of polyethylene glycol to simulate water stress showed that the fecundity, longevity, and reproductive period of the corn leaf aphid declined linearly with increased stress. It may well be that the corn leaf aphid ingest less sap per unit of time when the plants are stressed. In doing so, they also reduce their DIMBOA uptake, which make them appear less affected by the secondary metabolite, as shown in figure 2.2.

The limited set of data presented here suggests the following predictions about the operational resistance of maize to the corn leaf aphid in the field. In the absence of the resistance factor, DIMBOA, aphid populations would be subject to variations due to the stress status of the plant. At the other extreme, a high DIMBOA genotype appears to remain stable under varying growing conditions. A genotype intermediate in its level of DIMBOA also appears to be stable since the effects of plant stress and DIMBOA seem to balance each other (Fig. 2.2).

— Chapter III —

# DEVELOPMENT OF THE LADYBIRD *PROPYLEA QUATUORDECIMPUNCTATA* L. ON THE CORN LEAF APHID, *RHOPALOSIPHUM MAIDIS* FITCH., FROM STRESSED AND NON-STRESSED PLANTS OF RESISTANT AND SUSCEPTIBLE MAIZE INBREDS: EFFECTS OF PLANT ANTIBIOSIS ON A PREDATOR.

## 3.1 Introduction

One point of concern when breeding or transferring genes for plant resistance is how it may indirectly affect natural enemies. It has already been demonstrated that the use of chemical pesticides is a major disruptive factor of the action of beneficial insects (Stern *et al.*, 1959; Metcalf, 1982; Way and Heong, 1994) and, by the same token, plant resistance, achieved through artificial selection or by genetic engineering, may be incompatible with biological control (Boethel and Eikenbary, 1986; van Emden, 1995).

Resistance based on antibiosis (*sensu* Painter, 1951) is intuitively more likely to affect life history parameters of natural enemies in a negative way than antixenosis (*sensu* Kogan, 1982) or tolerance (*sensu* Painter, 1951). Orr and Boethel (1986) have shown this not only by demonstrating that soybean antibiosis can produce effects up to the fourth trophic level, but also by showing that the detrimental effects of plant antibiosis transferred through several physiological

systems. Indeed, plant antibiosis traveled up trophic levels *via* a caterpillar, an hemipteran predator, and ultimately manifested in a reduction of the fecundity of a parasitoid of the eggs of the predator, although emergence of the parasitoid remained unaffected.

The vast majority of laboratory investigations dealing with the repercussion of plant antibiosis upon the third trophic level focused on host-parasitoid systems (Campbell and Duffey, 1979; Philogène and Arnason, 1992). This may reflect the interest in the intimate nature of this type of association and the empirical and theoretical evidence that parasitoids are more effective biological control agents than predators (Beddington *et al.*, 1978). However, generalist predators and predators which specialize on a prey in a given crop, e.g. ladybird beetles on aphids, have been shown to sometimes contribute substantially to maintain crop pests under economic levels (Rice and Wilde, 1989; Ehler and Miller, 1978; Hagen *et al.*, 1976). Associated with partial levels of host plant resistance, generalist predators have complemented, or are believed to complement, the control of the pest complex of rice in southeast Asia (Pathak, 1977; Kartohardjono and Heinrichs, 1984).

There are only a few reported cases in which the life history parameters of a predator have been compared when fed prey from differentially resistant host plants (*see* Hare, 1992). Results from these studies are apparently context-specific and the basis for antibiosis are generally not known, contrasting with the detailed

investigations of plant secondary metabolites-host-parasitoid interactions (e.g., Campbell and Duffey, 1979; Campos *et al.*, 1990). Exception to this is the study by Martos *et al.* (1992) who examined life history parameters of the ladybird *Eriopis connexa* Germar fed aphids from wheat containing increasing concentrations of DIMBOA, a hydroxamic acid widely occurring in the Poaceae and implicated in the resistance to several insect pests and pathogens of wheat and maize (Niemeyer, 1988). Martos *et al.* (1992) found that larvae of *E. connexa* had the longest development time to pupation and the lowest survivorship when they ate aphids from the wheat line containing intermediate levels of DIMBOA. Their interpretation for this pattern derives from the deterrent property of high DIMBOA concentrations towards aphids which translates into reduced uptake of phloem sap and a decrease in the accumulation of the compound in the body of the aphids.

A similar investigation has been undertaken here, with larvae of the ladybird *Propylea quatuordecimpunctata* L. feeding on the aphid *Rhopalosiphum maidis* Fitch. from high DIMBOA and DIMBOA-deficient maize inbreds. In addition to resistance level, the present study evaluated the indirect effect of plant stress on developmental variables of the ladybird larvae. DIMBOA has been shown to be very sensitive to nutrient stress in maize seedlings (Manuwoto and Scriber, 1985a,b; Richardson and Bacon, 1993), and in late-whorl tissue (Chapter II), where *R. maidis* feeds in the early stage of infestation. Thus, plant stress may change the outcome, if any, of plant antibiosis on a predator.

## 3.1 Material and methods

### 3.2.1 Plant material and aphids

The seeds of the high DIMBOA inbred (A619) were obtained from Dr. R.I. Hamilton (Eastern Cereals and Oil Seed Research Centre, Central Experimental Farm, Agriculture Canada, Ottawa) and Dr. P. A. Peterson (Department of Agronomy, Iowa State University, Ames, USA) provided the seeds of the DIMBOA-deficient inbred (bxbx; original source: Dr. L. Gracen, Cornell University, Ithaca, USA).

The plants were grown in the field at the Central Experimental Farm (Agriculture Canada, Ottawa) at densities of 25 000/ha (no stress treatment) and 100 000/ha (stress treatment). Fertilizer (400 kg 18-18-18 N-P-K/ha) and herbicides (Round-up ® and Prime Extra ®) were applied at pre-planting. Three rows of high DIMBOA plants alternated with one row of low DIMBOA plants in plots of 7 rows. Plots of low planting density alternated with plots of high planting density. This pattern was repeated three times by sowing on 3 dates, 5 days apart. This not only ensured a sufficient supply of aphids for the whole course of the experiment, but also provided the ladybird larvae with aphids from colonies in their exponential growth phase, i.e. before the tassel becomes completely exposed and crowding

and/or a decrease in the nutritional value of the plant induce the declining phase of the colony.

### 3.2.2 Rearing of the ladybird larvae

Adults of *P. quatuordecimpunctata* were collected from the field and were sexed following Rogers *et al.* (1971). Mating pairs were kept in 75 ml vials and fed *R. maidis* from sorghum (*Sorghum vulgare;* Cargill hybrid, common soudangrass) growing in the field. After 24 hours, males were removed from the vials. Eggs were collected daily and kept in separate plastic boxes lined with humidified filter paper. When more eggs than needed for the experiment were laid in one day, they were isolated and monitored for hatching. At hatching, neonates were left on the egg masses for 24 hours so they could feed on unhatched eggs as they commonly do in the field (Osawa, 1989).

The 24-hour old larvae were assigned to the four treatments (aphids from DIMBOA-deficient and high DIMBOA inbreds, stressed or non-stressed), 15 larvae per treatment. The larvae were reared individually in 5 ml vials at 25 °C, 70 % r.h., and 16L:8D. New aphids were provided daily from the field grown plants. For each treatment, tassels from several plants were collected, brought to the lab, and vigorously shaken over a sheet of paper. Aphids were then easily transferred to

the rearing vials. The number of aphids provided to the ladybird larvae was in excess of their daily requirements.

The ladybird larvae were monitored twice daily for mortality and molting events. Only one larva per treatment died and, thus, mortality data will not be presented in the results section. One larva was also accidentally destroyed during manipulation. The duration of the larval stages (L1 through L4) and the pupal stages (pre-pupa and pupa) were separately analyzed by ANOVAs using half-day increment. Weighing of the individuals took place as soon as they emerged as adults and these data were also analyzed by ANOVA. Sex was considered as a factor in the statistical analyses.

### 3.2.3 Voracity of *P. quatuordecimpunctata*

Plant antibiosis can indirectly affect developmental variables of predators feeding on insect pests because the resistance factor travels up the food chain. However, development can also be altered if plant antibiosis leads to prey with reduced size (van Emden, 1995), because more prey would be needed to complete a predator's life cycle or because the resistance factor is deterrent to the predator. This was investigated.

At the time of the investigation, seeds of the DIMBOA-deficient maize inbred, bxbx, were not in my possession so maize genotypes that have been reported to be differentially resistant to *R. maidis* (A619 and W64A; Beck *et al.* [1983]) or to contain DIMBOA (BS9 C4; Bergvinson [1994]) were used. Seeds of these genotypes were provided by Dr. R.I. Hamilton (Eastern Cereals and Oil Seed Research Centre, Central Experimental Farm, Agriculture Canada, Ottawa).

Plants were grown in the greenhouse in 12 L pots (1 plant/pot) filled with a maize growth medium, watered using an automated system, and fed weekly (500 ml/pot of a solution made of 25 g of 20-20-20 N-P-K diluted in 1 L of tap water). At the late-whorl stage, the plants were inoculated with field-collected *R. maidis* from sorghum and covered with a sleeve-cage made of fine-mesh polyester. Ten days later, the aphids were brought to the laboratory and sorted into wingless adults and nymphs in their 3$^{rd}$ and 4$^{th}$ instars.

The immature aphids were offered to field-collected ladybird females previously starved for 24 hours, 50 per beetle, in a glass petri dish 15 cm in diameter. The space between the cover and the dish was packed with humidified cotton to contain the aphids. The number of aphids remaining after a period of 6 hours were counted. The experiment was conducted in an environmental chamber (25 °C, 70 % r.h.) with the lights on.

The number of replicates varied depending on the source of aphids since colonies were small on the most resistant plants. Nine replicates were performed with aphids from genotypes BS9 C4 and W64A, and 3 with aphids from A619. The mature aphids were weighed fresh and, again, the number of replicates depended on the genotype (38, 78, and 25 from BS9 C4, W64A, and A619 respectively). A Pearson's correlation analysis was performed to test whether a significant linear trend existed between the number of aphids consumed and their weight using mean values for both variables.

## 3.3 Results

### 3.3.1 Development time

The overall variation in the duration of the larval stages of *P. quatuordecimpunctata* in this experiment was very small (C.V. = 5.9 %; Table 3.1) and no significant differences could be detected among treatments or between sex for these life stages (Table 3.2). On the other hand, an increase in the duration of the pupal stages was observed for individuals of both sexes fed aphids from stressed plants of the high DIMBOA inbred (Table 3.3). Aphids from the high DIMBOA inbred did not, however, alter the duration of the pupal stages when the plants were not stressed. This difference in the response of the ladybirds is reflected in the significant inbred by plant stress interaction term (Table 3.4).

Table 3.1. Duration of larval stages of *P. quatuordecimpunctata* fed corn leaf aphids from stressed and non-stressed plants of DIMBOA-deficient (bxbx) and high DIMBOA (A619) maize inbreds.

| Sex | stress level on plants | aphid source | | | |
|---|---|---|---|---|---|
| | | bxbx (DIMBOA-deficient) | | A619 (high DIMBOA) | |
| | | n | days (SE) | n | days (SE) |
| males | non-stressed | 9 | 7.17 (0.08) | 6 | 7.25 (0.25) |
| | stressed | 4 | 7.63 (0.47) | 8 | 7.31 (0.09) |
| females | non-stressed | 5 | 7.20 (0.12) | 7 | 7.36 (0.21) |
| | stressed | 10 | 7.25 (0.25) | 6 | 7.25 (0.11) |

Table 3.2. Analysis of variance on larval development time data.

| Sources of variation | *d.f.* | *M.S.* | *F* | *p* |
|---|---|---|---|---|
| inbred | 1 | 0.004 | 0.02 | 0.8821 |
| stress | 1 | 0.170 | 0.92 | 0.3420 |
| sex | 1 | 0.070 | 0.38 | 0.5416 |
| inbred * stress | 1 | 0.242 | 1.31 | 0.2581 |
| inbred * sex | 1 | 0.118 | 0.64 | 0.4279 |
| stress * sex | 1 | 0.265 | 1.43 | 0.2375 |
| inbred * stress * sex | 1 | 0.045 | 0.24 | 0.6235 |

Table 3.3 Duration of pupal stages (pre-pupa and pupa) of *P. quatuordecimpunctata* fed corn leaf aphids from stressed and non-stressed plants of DIMBOA-deficient (bxbx) and high DIMBOA (A619) maize inbreds.

| Sex | stress level on plants | aphid source | | | |
|---|---|---|---|---|---|
| | | bxbx (DIMBOA-deficient) | | A619 (high DIMBOA) | |
| | | n | days (SE) | n | days (SE) |
| males | non-stressed | 9 | 5.28 (0.15) | 6 | 5.33 (0.25) |
| | stressed | 4 | 5.00 (0.35) | 8 | 5.56 (0.18) |
| females | non-stressed | 5 | 5.40 (0.19) | 7 | 5.29 (0.18) |
| | stressed | 10 | 5.33 (0.25) | 6 | 5.67 (0.11) |

Table 3.4 Analyses of variance on pupal development time data.

| Sources of variation | *d.f.* | *M.S.* | *F* | *p* |
|---|---|---|---|---|
| inbred | 1 | 0.908 | 4.51 | 0.0391 |
| stress | 1 | 0.001 | 0.00 | 0.9491 |
| sex | 1 | 0.062 | 0.31 | 0.5830 |
| inbred * stress | 1 | 1.118 | 5.55 | 0.0227 |
| inbred * sex | 1 | 0.022 | 0.11 | 0.7440 |
| stress * sex | 1 | 0.013 | 0.07 | 0.7894 |
| inbred * stress * sex | 1 | 0.024 | 0.12 | 0.7316 |

### 3.3.2 Adult weight

Females weighed significantly more than males but, overall, adult weight was not significantly affected by aphid source (Tables 3.5 and 3.6). Though, an almost significant higher order interaction was found between inbred, stress level, and sex (Table 3.6). This may be due to the female response going opposite to the general trend for adult weight to decrease when, as larvae, they fed on aphids of the high DIMBOA inbred or from stressed plants (Table 3.5).

### 3.3.3 Voracity of *P. quatuordecimpunctata* females

There was a negative relationship between the mean number of immature aphids eaten by female beetles and the mean body mass of mature aphids from different maize genotypes. Though significant only at $p = 0.066$ (n= 3, $r_{Pearson} = -0.995$), there was a trend for female beetles to consume more of smaller aphids (Fig. 3.1). The values of the cross-product of the mean number of immature aphids eaten and the mean body mass of mature aphids are similar for each maize genotype (A619 = 21.30 mg, W64A = 21.95 mg, and BS9 C4 = 21.79 mg), suggesting that no deterrent effect was operating.

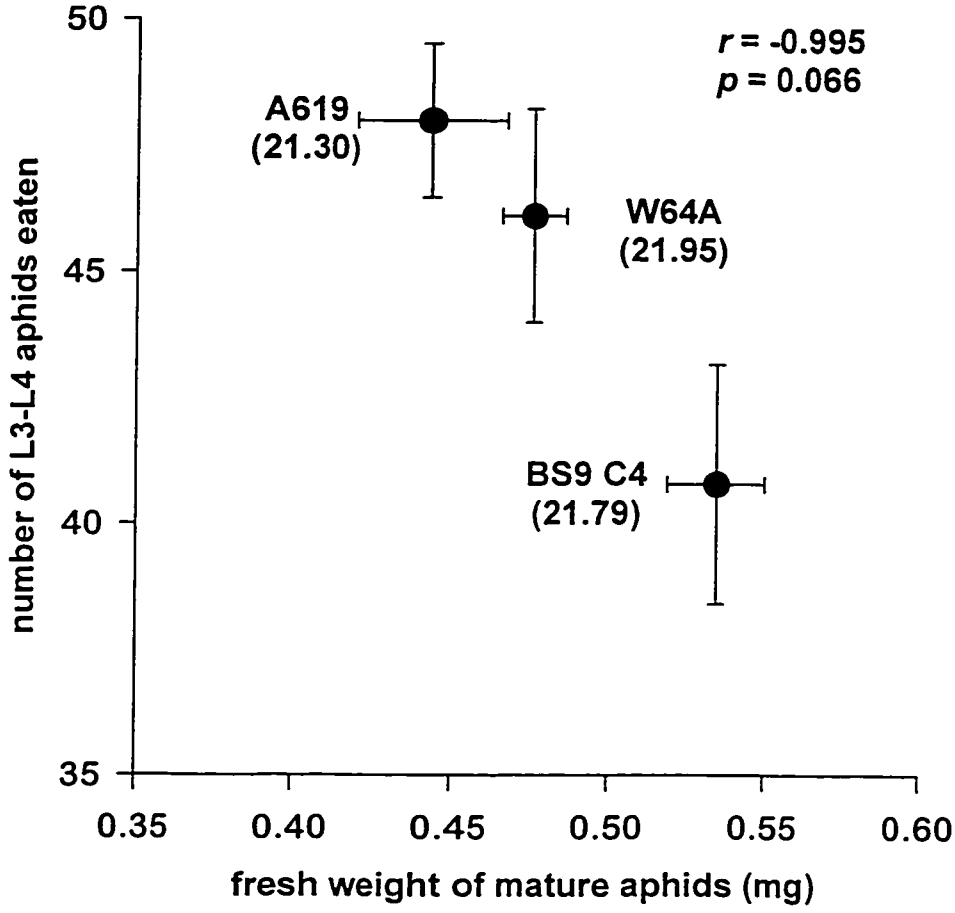
Table 3.5 Weight of adults of *P. quatuordecimpunctata* fed corn leaf aphids from stressed and non-stressed plants of DIMBOA-deficient (bxbx) and high DIMBOA (A619) maize inbreds during their larval stages.

| Sex | stress level on plants | aphid source | | | |
|---|---|---|---|---|---|
| | | bxbx (DIMBOA-deficient) | | A619 (high DIMBOA) | |
| | | n | days (SE) | n | days (SE) |
| males | non-stressed | 9 | 7.48 (0.20) | 6 | 7.38 (0.20) |
| | stressed | 4 | 7.37 (0.21) | 8 | 7.20 (0.41) |
| females | non-stressed | 5 | 8.82 (0.31) | 7 | 8.18 (0.25) |
| | stressed | 10 | 8.43 (0.14) | 6 | 9.19 (0.33) |

Table 3.6 Analysis of variance on adult weight data.

| Sources of variation | *d.f.* | *MS* | *F* | *p* |
|---|---|---|---|---|
| inbred | 1 | 0.0002 | 0.13 | 0.7216 |
| stress | 1 | 0.0001 | 0.06 | 0.8097 |
| sex | 1 | 0.0640 | 42.97 | 0.0001 |
| inbred * stress | 1 | 0.0030 | 2.00 | 0.1635 |
| inbred * sex | 1 | 0.0004 | 0.29 | 0.5897 |
| stress * sex | 1 | 0.0021 | 1.39 | 0.2452 |
| inbred * stress * sex | 1 | 0.0048 | 3.24 | 0.0782 |

Figure 3.1. Voracity of *P. quatuordecimpunctata* female beetles on immature *R. maidis* (L3-L4) from different maize genotypes in relation to aphid body mass (measured on wingless adults). The beetles were initially offered 50 aphids and the experiment lasted 6 hours. Means and standard errors are shown. The values in brackets are the cross-product of the mean number of aphids eaten and the mean body mass of aphids from the same genotype (expressed in mg).


50
45
40
35
number of L3-L4 aphids eaten
r = -0.995
p = 0.066
A619
(21.30)
W64A
(21.95)
BS9 C4
(21.79)
0.35
0.40
0.45
0.50
0.55
0.60
fresh weight of mature aphids (mg)

## 3.4 Discussion

The results of this study show that there are no statistically detectable indirect effects of DIMBOA-based plant resistance or plant stress on the duration of the larval stages (Tables 3.1 and 3.2) or on adult weight (Tables 3.5 and 3.6) of the ladybird *P. quatuordecimpunctata* feeding on the aphid *R. maidis*. Survivorship to adulthood was also unaffected (93.3 % across all treatments). The duration of the combined pre-pupal and pupal stages was, however, significantly increased when the larvae were fed *R. maidis* from stressed plants of the high DIMBOA inbred (Tables 3.3 and 3.4). Resistance level did not lead to this increase in pupal duration when the plants were not stressed (Tables 3.3 and 3.4).

Although the indirect effect of plant stress on an aphid predator is, to my knowledge, unprecedented, the results presented here contrast markedly with other reported effects of a host plant resistance mediated alteration of life-history variables of a ladybird beetle. DIMBOA-based resistance in wheat has been reported to increase larval development period, but not pupal development period, of the ladybird *Eriopis connexa* feeding on the aphid *R. padi* (Martos *et al.*, 1992). Rice and Wilde (1989) also reported an increase in larval time of the ladybird *Hippodamia convergens* feeding on the aphid *Schizaphis graminum* from two resistant sorghum hybrids, but they did not evaluate pupal development period. Host plant resistance also mediated a reduction in the survivorship of the ladybirds in the

studies by Martos *et al.* (1992) and Rice and Wilde (1989), but this was not observed here. Martos *et al.* (1992) did not measure ladybird body mass in their study and Rice and Wilde (1989) did not find a significant mediated effect of host plant resistance on this variable, although they have found a significant interaction between resistance level and sex, with males weighing more when fed aphids from the resistant sorghum hybrids while females weighed less. Here, although not significant, males tended to weigh less when fed aphids from the high DIMBOA maize inbred, this difference being somehow magnified when plants were stressed (Table 3.5). Females showed the same trend, except when fed aphids from stressed plants of the high DIMBOA maize inbred, in which case they tended to weigh more.

The discrepancies between the results presented here and the other published results should be weighed in relation to the magnitude of the indirect effects of the plant on the developmental variables of the ladybird beetles studied. In particular, the reported increases in development time, though significant, are less than one day. Whether an increase of such small magnitude is important in natural populations of ladybird beetles is not known. It should be stressed, however, that the effects of a lengthening of the immature stages are likely to be different depending on which particular stage is affected, larval or pupal, because different risks are involved. An increase in the duration of the feeding stages augments the risk that a ladybird larva cannot complete its development during

the existence of an aphid colony (Hemptinne *et al.*, 1992). An increase in the duration of the sessile pupal stages augments the risk of generalist predation, intraguild predation, or cannibalism. The relative importance of these stage-specific risks should be assessed in relation to host-plant resistance, and more specifically, to the densities of both the predator and the prey as mediated by resistance level.

In itself, low prey density, as mediated by plant resistance, is likely to increase the risk of a ladybird larva not completing its development within the duration of an aphid colony or of an episode of infestation. An increase in the duration of the immature feeding stages would thus magnify that risk. Another factor involved in the risk a ladybird larva faces in relation to plant resistance is the reduced size of aphids on resistant plants (van Emden, 1995). This means that, on resistant plants, more prey would be needed for the ladybird larva to complete its development. Figure 3.1 demonstrates this point by showing that the number of aphids consumed by a ladybird beetle is negatively related to aphid size, though the correlation was of borderline significance due to the limited number of replicates performed (n = 3). Because aphids on resistant plants are already scarce, reduced aphid size also means that, once an aphid colony is found by a foraging ladybird larva, there is an additional risk that there is not enough aphids to satisfy its requirement. Gilbert *et al.* (1976) have shown that when aphids are scarce on alfalfa, ladybird beetles do not have enough time to find all the prey they need.

Hence, all the risks inherently associated with low aphid density are likely to be magnified when the indirect effect of plant resistance results in an increase of the immature feeding stages of a ladybird beetle, even if small. On the other hand, the predation risks associated with an extended ladybird pupal development period should not be important on resistant plants since predators are also likely to be scarce. However, a small increase in the duration of a vulnerable stage may become increasingly important with the ladybird reproductive response to aphid density (Mills, 1982). Osawa (1993) has shown, using key-factor analysis of field populations of *Harmonia axyridis*, that pupal mortality due to cannibalism was density-dependent. Though not significant, the ladybird pupal times measured in the present study tended to increase with plant stress. Clearly, a detailed field study would be needed to specifically address the question of this unexpected indirect plant effect on the third trophic level.

— Chapter IV —

# Evidence for Spatial Niche Partitioning in Predaceous Aphidophaga: Use of Plant Color as a Cue

## 4.1 Introduction

Direct and indirect effects of plants on the third trophic level must be investigated to fully understand tritrophic interactions (Vinson, 1976; Price *et al.*, 1980; Boethel and Eikenbary, 1986). Several studies have shown that natural enemies use plant-based cues, e.g., plant volatiles, to locate their prey (Powell and Zhi- li, 1983; Nordlund *et al.*, 1988; Whitman and Eller, 1992; Braimah and van Emden, 1994). However, parasitoids rather than predators have been the focus of such studies and little is known about the foraging behavior of adult predaceous aphidophaga (Ferran and Dixon, 1993). In particular, virtually nothing is known about habitat location by aphidophagous predators and, more specifically, whether they use plant cues to locate their prey. After all, predaceous aphidophaga face the same problems as parasitic aphidophaga when invading temporary habitats such as agricultural fields to find prey.

In this study, I was interested to determine if plant cues are used by predaceous aphidophaga when they invade maize fields. This is part of a larger study on the effects of plant stress and plant resistance on the biological control of

the corn leaf aphid, *Rhopalosiphum maidis*. Because nutrient stress results in plants with a generally lighter color (yellow) than well fertilized plants, I focused primarily on plant color as the discriminating factor for aphid predators to respond to. By examining this single factor, I wanted to see if predaceous aphidophaga belonging to different taxa responded differentially. To avoid the confounding effects of the presence of aphids on the plants on the predators' response, the plants were grown in a greenhouse and moved to the field before *R. maidis*, the most important aphid species on maize in Canada (Coderre, 1981; 1983), migrates from the south. The eggs and the larvae of the predators were removed daily to minimize density-dependent effects on the adults (Hemptinne *et al.*, 1993; Růžička 1994; 1996).

## 4.2 Material and methods

### 4.2.1 Plants

Plants from 5 maize genotypes differentially resistant to *R. maidis* were used in this experiment. From the most resistant to the most susceptible, these genotypes were: CO273 (hereafter C), A619 (A), W64A (W), BS9C4 (B), and bxbx (X). All are inbreds, except genotype B which is a synthetic. The plants were started in a greenhouse in May 1995. Seeds were sown in 12 L pots filled with a

maize soil medium used at the Central Experimental Farm, Ottawa (Agriculture Canada). Water was provided through an automatic watering system and fertilizer was applied once a week (500 ml/pot of a solution made of 25 g of 20-20-20 N-P-K diluted in 1 L of tap water). For the stress treatment, 3 plants were grown in the same pot, whereas control plants were grown singly. Plants were moved to the field when they reached the late whorl stage, i.e., when *R. maidis* normally colonizes maize. For each maize genotype, 3 control plants of similar size and form to the stressed plants were selected. Each group of 3 plants was then randomly placed in a hole lined with a plastic sheet and half-filled with sand. The plants within each group were equally spaced. Holes were 1.5 m apart and were dug to form a grid of 8 by 10. The ground was kept barren around the holes. There were eight replicates for each combination of maize genotype and stress/control treatments. Plants were allowed to acclimatize for 5 days before counts of predators started. During this period and for the rest of the experiment, plants were watered and fertilized.

### 4.2.2 Color rating of the plants

A relative method of scoring the color of the plants was used in this experiment. Six observers, with no prior knowledge of the purpose of the experiment and unfamiliar with the maize genotypes chosen for this study, were asked to rate each group of 3 plants on a scale of 1 (=yellow) to 5 (=green). The

reference groups of plants for the extreme values of the scale were part of the experimental plants and were indicated to the observers by me. A two-factorial ANOVA with interaction was performed on the rating data and the mean color rating for each combination of maize genotype and stress/control treatment was used in Pearson's correlation analyses with the mean cumulative number of predators (SAS, 1987).

### 4.2.3 Counts of predators

Plants in the field were examined for predators during four consecutive days. Counts took place between 8 and 12 AM and were made by two observers. Each group of three plants was searched for 5 minutes. Immature stages (eggs and larvae) were removed. The presence of *R. maidis* was also recorded. Aphids were seen only on the last day, with no more than 5 aphids/group counted on the 18 groups of plants (out of 79[2]) where they were observed. ANOVAs were performed on pooled counts of immature and mature stages of predators made over the 4 days the experiment lasted. Separation of means was accomplished by the SNK method (SAS, 1987).

[2]One group of 3 plants was dropped from the experiment because the stems were broken by the wind.

## 4.3 Results

### 4.3.1 Color rating of the plants

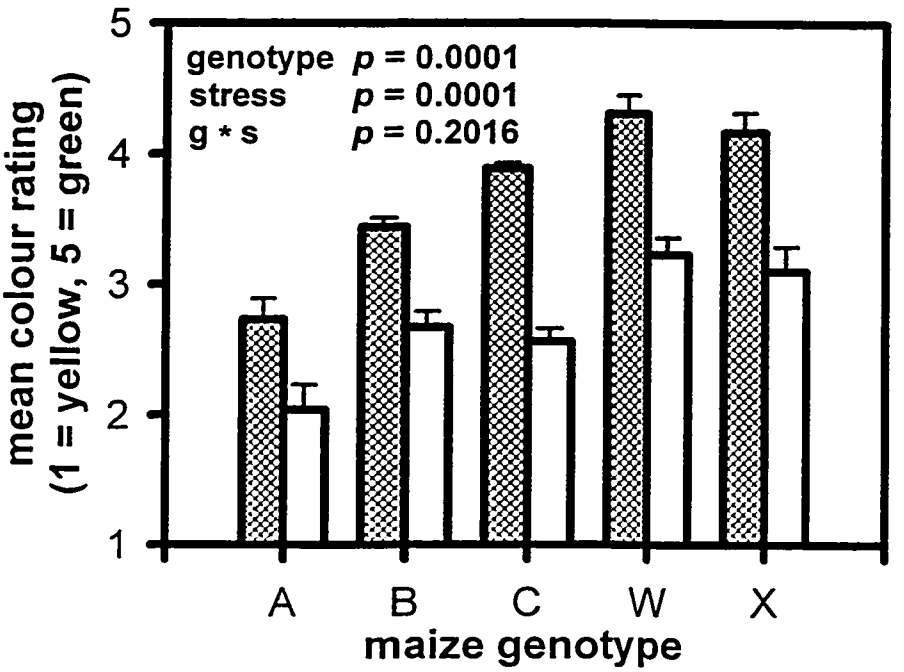
The relative color rating method used in this study proved sensitive enough (Fig. 4.1) to discriminate control from stressed plants, the latter being significantly lighter in color (*d.f.* = 1, 69; $F = 114.46$; $p = 0.0001$), and to find significant differences between maize genotypes (*d.f.* = 4, 69; $F = 28.34$; $p = 0.0001$). The interaction term in this analysis was not significant (*d.f.* = 4, 69; $F = 1.54$; $p = 0.2016$).

### 4.3.2 Counts of predators

Only members of the Coccinellidae and the Chrysopidae were found on the experimental plants. Syrphid flies were seen within the experimental plot and sometimes hovered near the plants, but none were seen to forage or to have left evidence of their presence, e.g., eggs or larvae.

A total of 131 adult ladybird beetles were counted over the four days of the experiment. They were seen walking on the plants, resting, or mating. Four larvae and one pupa were also seen, but were not included in the cumulative count because no eggs were laid by the beetles and, hence, it was assumed that their presence on the plants was the result of fortuitous encounters.

Figure 4.1. Means and standard errors of color rating of the maize genotypes used in this study. Filled bars: control plants. Empty bars: stressed plants.

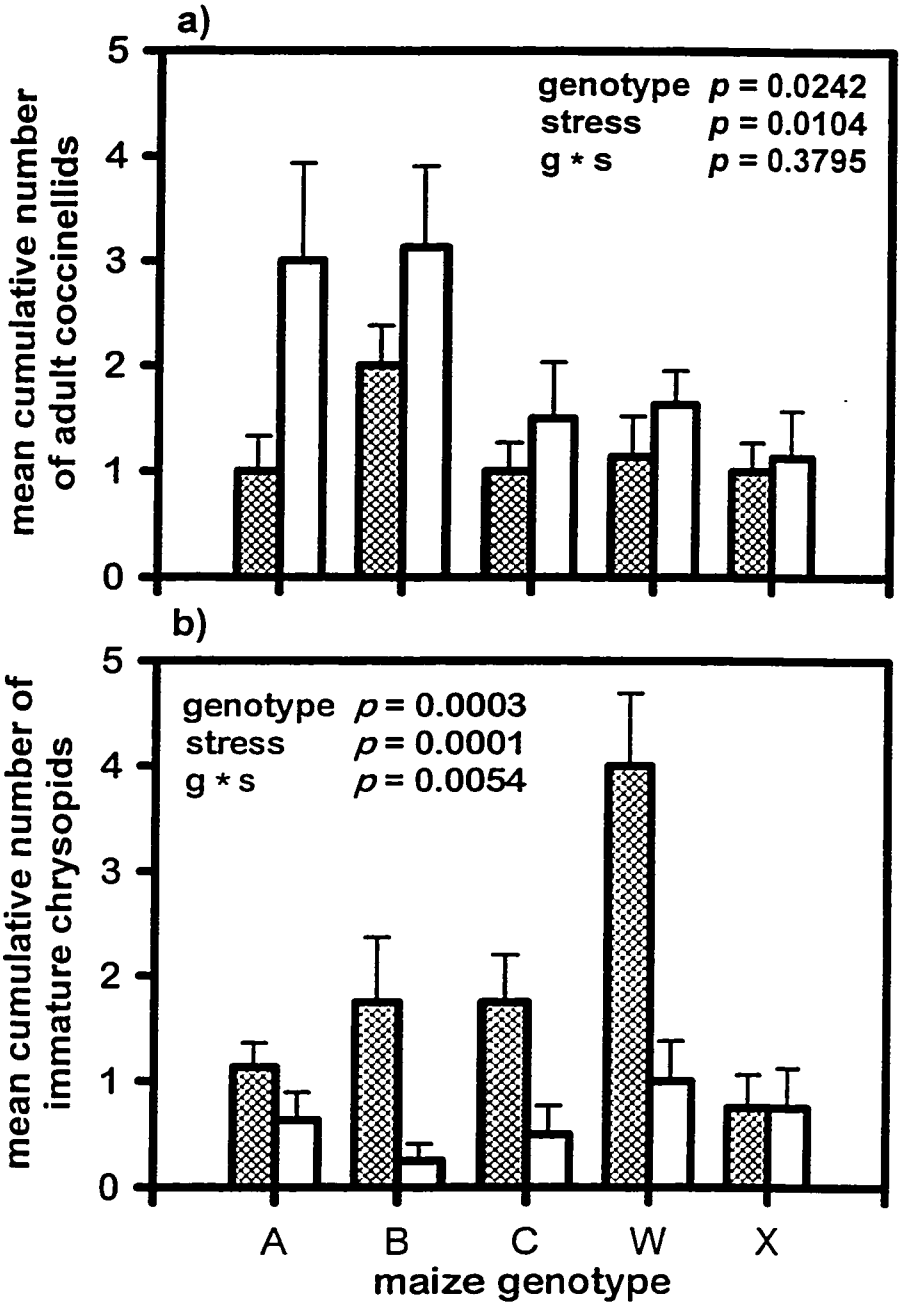

mean colour rating
(1 = yellow, 5 = green)
5
4
3
2
1
genotype p = 0.0001
stress p = 0.0001
g * s p = 0.2016
A
B
C
W
X
maize genotype

The species of ladybird beetles observed were: *Propylea quatuordecimpunctata* L. (n = 63), *Coleomegilla maculata lengi* Timberlake (n = 35), *Coccinella septempunctata* L. (n = 16), *Adalia bipunctata* (L.) (n = 7), *Hippodamia tredecimpunctata tibialis* Say (n = 5), *Coccinella trifasciata perplexa* Mulsant (n = 2), and *Hippodamia convergens* Guérin (n = 1). Two individuals could not be identified.

Significantly more coccinellid adults occurred on the stressed than on the control plants (Fig. 4.2a; *d.f.* = 1, 69; $F = 6.94$; $p = 0.0104$). No significant interaction was found between maize genotype and stress level (*d.f.* = 4, 69; $F = 1.07$; $p = 0.3795$). Maize genotypes were not equally preferred by ladybird adults (*d.f.* = 4, 69; $F = 3.00$; $p = 0.0242$), with plants of genotype B being significantly more visited (SNK; $p = 0.05$) than plants of the other genotypes, although many coccinellid adults were seen on stressed plants of genotype A (Fig. 4.2a).

No adult chrysopids were seen on the experimental plants, but eggs (n = 58) and young larvae (n = 38) were present. The number of eggs found increased from day 1 to day 4 while the number of larvae found decreased, indicating that female chrysopids started to forage before the beginning of the experiment and that eggs and larvae were effectively removed at each count.

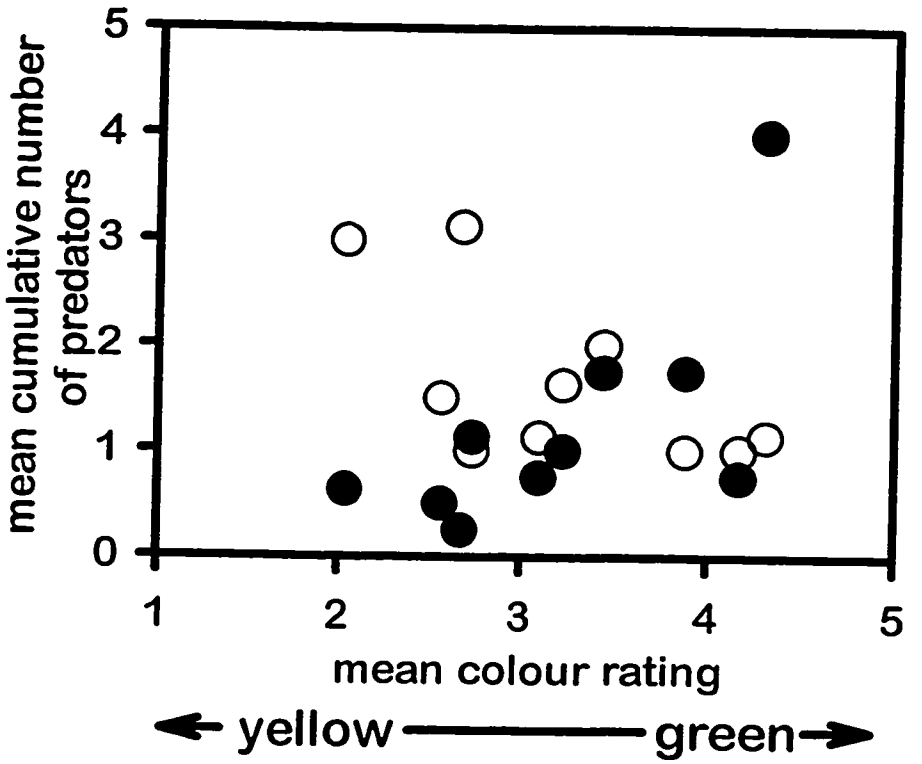
Figure 4.2. Means and standard errors for the cumulative counts over a period of 4 days of (a) adult coccinellids and (b) immature chrysopids on control (filled bars) and stressed (empty bars) plants of different maize genotypes.


a)
mean cumulative number of adult coccinellids
genotype p = 0.0242
stress p = 0.0104
g * s p = 0.3795
b)
mean cumulative number of immature chrysopids
genotype p = 0.0003
stress p = 0.0001
g * s p = 0.0054
A
B
C
W
X
maize genotype

Independent ANOVAs were performed for chrysopid data because a significant maize genotype by stress level interaction was found (*d.f.* = 4, 69; $F$ = 4.03; $p$ = 0.0054). Contrary to the pattern observed for coccinellids, chrysopids (Fig. 4.2b) preferred controls over stressed plants of genotypes B, C, and W (all differences significant at $p$ = 0.05). No significant differences in the cumulative number of chrysopid immature stages were found for plants of genotypes A and X. Among controls, plants of genotype W were significantly preferred over plants of the other genotypes (*d.f.* = 4, 34; $F$ = 6.42; $p$ = 0.0006; SNK, $p$ = 0.05). No differences were found among the stressed plants (*d.f.* = 4, 35; $F$ = 0.88; $p$ = 0.4862).

The attraction of coccinellids and chrysopids for all the different combinations of maize genotypes and stress level showed opposite trends (Fig. 4.3), but not significantly so (n = 10; $r_{Pearson}$ = -0.39; $p$ = 0.267). However, figure 4.3 shows that the mean cumulative numbers of adult coccinellids significantly decreased with the mean color rating of the plants, i.e., from yellow to green (n = 10; $r_{Pearson}$ = -0.64; $p$ = 0.048), while the mean cumulative numbers of immature stages of chrysopids significantly increased (n = 10; $r_{Pearson}$ = 0.67; $p$ = 0.034).

Figure 4.3. Mean cumulative number of predators found on plants of all the different combinations of maize genotypes and stress levels in relation to the mean color rating of the same plants (see figure 4.1). Open circles: coccinellids. Closed circles: chrysopids.


mean cumulative number of predators
0
1
2
3
4
5
1
2
3
4
5
mean colour rating
yellow
green

## 4.4 Discussion

In the absence of aphids, both coccinellids and chrysopids were significantly attracted to plants of specific maize genotypes, but this attraction depended on the level of stress the plants experienced (Fig. 4.2a+b). Coccinellids were more attracted to stressed plants of all maize genotypes, which were also lighter in color than control plants (Fig. 4.1), whereas the opposite pattern was observed for chrysopids, at least in 3 out of the 5 maize genotypes included in this study. Adult coccinellids were more abundant on plants of genotype B and stressed plants of genotype A, although not significantly so in the latter case, than on plants of other genotypes. Immature stages of chrysopids were more abundant on control plants of genotype W, but this preference disappeared when the plants were stressed.

The overall pattern of attraction of coccinellids and chrysopids to different maize plants, stressed or not, is better seen and understood when the mean number of predators observed on the plants is plotted against the mean color rating of the same plants (Fig. 4.3). There was a significant decrease in the number of coccinellids observed on the plants as the greenness of the leaves increased whereas the number of chrysopids increased significantly on the same scale. Although this color scale was an artificial one, these results suggest that the response of coccinellids and chrysopids was related to the intensity of green of the plants used in this study rather then associated to some specific of a given maize

genotype. The fact that attractiveness for plants of a particular maize genotype depended on the level of stress imposed, which resulted in a variation in the intensity of green (Fig. 4.1), is another indication that the overall response of the predators was related to plant color itself. In other words, different combinations of maize genotype and stress level scored the same on the color scale (Fig. 4.1), and predators responded to the color of the plants in these combinations (Fig. 4.3) and not specifically to the level of stress or to a particular genotype. To our knowledge, this is the first documented evidence that aphid predators, in particular coccinellids and chrysopids, appear to be attracted to plant color.

At this point it is not possible to ascertain that coccinellids and chrysopids, or any insect for that matter, perceive colors as humans do (Prokopy and Owens, 1983), although some studies have indicated that parasitoids seem to respond to color (*see* Obrycki, 1986) and Ricci (1986) reported that coccinellids are effectively caught in yellow traps. It is worth noting that the pattern of plant selection reported here for coccinellids, which were more attracted to yellow plants, and for chrysopids, which were more attracted to green plants, is consistent with the color of the eggs they lay and the crypsis that results from laying eggs on plants of the right color: yellow eggs for coccinellids and pale green eggs for chrysopids. However appealing this observation may be, egg crypsis cannot be the only explanation for the pattern reported here because the question of why coccinellids

evolved to produce yellow eggs and chrysopids pale green eggs remains unanswered.

What would be the benefit to coccinellids and chrysopids to use plant color as a cue? The absence of a significant correlation between the number of individuals observed in the two groups of aphidophaga, which respond in opposite way to color variation, suggests that this pattern is not the consequence of an interaction in ecological time, though individuals, especially larvae, may interact negatively when there are encounters (Lucas *et al.*, 1997). Rather, the hypothesis we propose to account for the attraction of coccinellids and chrysopids for plants of different color is that this behavior has evolved to minimize intraguild interactions. By specializing on aphids, which develop synchronous colonies within a given habitat (Dixon, 1985), aphidophaga cannot avoid the temporal constraints tied to the precarious resource they exploit: they have to feed and reproduce in a narrow window of time (Hemptinne *et al.*, 1992). However, by using plant cues such as plant color, aphidophaga belonging to different taxa can use sub-units of the same habitat simultaneously, thus minimizing intraguild interactions.

Although the spatial niche-partitioning hypothesis we propose for coccinellids and chrysopids requires further confirmation (Connell, 1980), the results presented here are different from other studies that have shown direct plant

effects on the foraging success of aphid predators or parasitoids, e.g., waxy leaves, foliar pubescence, or presence of glandular trichomes (Banks, 1957; Carter *et al.*, 1984; Obrycki, 1986). Spatial distribution and segregation of species of aphid predators have, however, already been reported. Honek (1985) has shown that different species of coccinellids are attracted to different plant forms and Coderre *et al.* (1987) have shown that there is a vertical distribution of coccinellids species on tall plants such as maize. However, this study is the first to show evidence of niche partitioning between two co-occurring groups of predaceous aphidophaga, based on plant color. The use of the information presented here in the context of biological control is not straightforward as it reveals another aspect of the complexity of tritrophic interactions.

## — Chapter V —

# PLANT RESISTANCE AND LADYBIRD BEETLES IN A MAIZE AGRO-ECOSYSTEM: WINDOW FOR STABILITY AT THE THIRD TROPHIC LEVEL

## 5.1 Introduction

Pioneer workers in the area of plant resistance, such as Painter (1951), envisioned the possibility of combining this strategy to control insect pests with the use of biological control agents. van Emden and Wearing (1965) were probably the first to give some theoretical consideration to this matter. They argued that host-plants with a moderate level of resistance, by reducing the rate of increase of insect pests, would give natural enemies the edge required to repress the growth of their prey. The experimental evidence in agreement with this prediction came later from the work of Starks *et al.* (1972) on aphids feeding on partially resistant and susceptible barley hybrids. Resistance alone was insufficient to provide acceptable control, whereas the addition of an aphid parasitoid kept the aphids in check on the partially resistant hybrid.

Partial host-resistance has many advocates (Dodd, 1973; van Emden, 1986; Obricky, 1986). These proponents of the limited expression of host-resistance consider agro-ecosystems developed around this strategy to be more stable than systems in which the highest level of resistance is implemented. However, the aim

is generally to achieve the maximization of predation or parasitism rates, hoping for equilibrium pest densities to be below economic threshold levels. I argue here that high predation or parasitism rates may sometimes be transient dynamics in the system under study. Unless the response of natural enemies to a wide range of prey densities is known, predictions about stability of the interaction cannot be safely made. This is particularly true for predators with generation times much larger than their prey. The ladybird beetle-aphid interaction is a good example of such. At the landscape level, populations of both aphids and their ladybird predators undergo large oscillations in their numbers dynamics (Hodek, 1970; Dixon, 1975; Kindlmann and Dixon, 1993). This may explain why sometimes aphidophagous ladybird beetles are reported to be effective biological control agents, and also why they sometimes failed dramatically at this task.

In the present study, the focus was on stability at the third trophic level. This was examined by looking at the variation in the survivorship of the immature stages of a natural enemy in response to a range of prey densities generated by host-plants expressing different levels of resistance. It was hypothesized that survivorship would gradually increase as the number of prey available increases, and then level-off with further increases in prey density since the reproductive response of predators must saturate at some point (Holling, 1961). Since the reproductive response saturates, there will be no accrued benefit from increasing prey densities to the natural enemy. Hence, the prey density at which a plateau is

reached in terms of survivorship of the immature stages of the predator would define the conditions which lead to stability at the third trophic level, i.e. a window mediated by the amount of plant resistance in the system.

The maize-aphid-ladybird system is particularly appropriate for such a study. The corn leaf aphid *Rhopalosiphum maidis* is the dominant species in this system in Canada (Foot, 1977; Coderre, 1981, 1983). It migrates each year from the south and infestations are typically initiated at the late-whorl stage, while the tassel has not yet emerged. Aphid colonies become extinct as the tassel matures and shed pollen. A second stage of infestation follows within the husks in which aphids enjoy an absolute refuge from their main predators in this system, i.e. ladybird beetles (Coderre and Tourneur, 1986). Hence, this system can be studied within well-defined temporal boundaries, i.e. the maturation of the tassel. In addition, ladybird beetles increase numerically only as the tassels are about to emerge from the whorl (Wright and Laing, 1980). This particularity greatly simplifies the estimation of available prey to the ladybird larvae since peak aphid number can be used directly as the independent variable.

Previous work on maize resistance to *R. maidis*, based on the secondary metabolite DIMBOA, has provided a large inventory of germplasm from which to pick from to generate the widest range of aphid density possible, including the DIMBOA-deficient inbred bxbx (Long *et al.*, 1977; Beck *et al.*, 1983). Since DIMBOA

has been reported to be sensitive to plant stress (Manuwoto and Scriber, 1985a,b; Richardson and Bacon, 1993), treatments such as increasing plant density and nitrogen rate have also been implemented in this study. Not only the sensitivity of DIMBOA to plant stress was expected to generate more variation in the number of aphids available to the ladybirds, but stress treatments would also provide a more general test for the stability hypothesis at the third trophic level which is proposed here.

## 5.2 Material and methods

### 5.2.1 Plant material

In 1994 and 1995, 3 differentially resistant maize inbreds were planted in the field, including the DIMBOA-deficient bxbx (obtained from Dr. P. A. Peterson, Department of Agronomy, Iowa State University, Ames, USA; original source: Dr. L. Gracen, Cornell University, Ithaca, USA), the partially resistant W64A (Beck *et al.*, 1983), and the highly resistant CO273 (as established in a previous preliminary field experiment using caged plants inoculated with the aphid *R. maidis*).

In 1996, 3 differentially resistant maize genotypes were also planted, but included the DIMBOA-deficient bxbx inbred, the high-DIMBOA inbred A619Ht

(Chapter II), and the cross (A619Ht x bxbx) which is intermediate with respect to DIMBOA content (Chapter II). The seeds of the high DIMBOA inbred (A619Ht) were obtained from Dr. R.I. Hamilton (Eastern Cereals and Oil Seed Research Centre, Central Experimental Farm, Agriculture Canada, Ottawa). The cross (A619Ht x bxbx) was performed in the greenhouses of the Central Experimental Farm, Agriculture Canada, Ottawa.

### 5.2.2 Experimental designs

All experiments reported here were performed in the same field at the Central Experimental Farm, Agriculture Canada, Ottawa. In addition to resistance level, plant stress was a factor included in all experiments. In 1994 and 1995, three levels of plant density were performed as a mean of stressing the plants: 25, 50, and 100 thousand plants per hectare. These levels represent a trend encompassing cultural practices for maize from the early 1900's to the expected density at the turn of the century. The distance between the plants in a row were 42.5 cm (low density), 20.0 cm (intermediate density) and 12.5 cm (high density).

Each experimental plot consisted of 5 rows, 3.5 m in length and 80 cm apart, with the plants in the middle row being the experimental plants (except plants at each end of the row) on which all data were collected, unless stated otherwise (*see later*). Because it was not known if, and how, plant density would affect selection

by aphids and ladybird beetles, the experimental designs in 1994 and 1995 were split-plots, with plant density as the main plots and maize inbred as the sub-plots. Each treatment was replicated 4 times in separate blocks. Within each block, strips of main plots were separated by 3.5 m of bare ground. Within each strip of main plots, sub-plots were contiguous. To minimize migration of ladybird larvae between sub-plots, the plants in the first and fifth rows in each sub-plot were stripped of their leaves at the late whorl stage and the budding tassels were severed. Also, a thick coat of Tangle Foot® was applied on the first 15 cm of the stem of each plant in these border rows. It was observed that ladybird larvae were repelled by the product.

Enclosing each block, two rows were planted with a mixture of commercial hybrids with different maturity, most of them being earlier than the experimental genotypes. Surrounding the whole experimental field, sorghum (*Sorghum vulgare;* Cargill hybrid, common soudangrass), which is colonized at the seedling stage by *R. maidis*, was planted. This ensured that *R. maidis* would build-up large populations in the area of the experimental field by the time the experimental plants would be at the appropriate stage for the initiation of colonies.

Planting of experimental and non-experimental plants occurred on May 24$^{th}$ of each year, except for the inbred CO273 which was planted a week later since it matures earlier than the two other inbreds experimented. Fertilizer (400 kg/ha

18-18-18 N-P-K) and herbicides (Round-up® and Prime-Extra®) were applied only at pre-planting with farm machinery operated over the whole field. Weeding after emergence was done by hand (between rows) or with a small roto-tiller (around plots). The importance of keeping the ground completely barren should be emphasized for such experiments since Coderre *et al.* (1987) have shown that there is a vertical segregation among species of ladybird beetles for oviposition sites on tall plants such as maize. It was also observed in a preliminary experiment that weeds were often selected as oviposition sites, probably even preferred over the maize plants by some species of ladybird beetles. Because of this, and to keep the number of stems in the experimental plots similar with respect to plant density (since the number of tillers increases with increasing space between plants), maize tillers were also severed.

Both the experimental design and the experimental plant material for the field experiment performed in 1996 were modified for two reasons. First, it was found from the previous experiments that plant density in itself, in particular low and intermediate densities, may have been a factor affecting the foraging success of ladybird larvae, confounding the effect of plant stress (*see the Results section*). Consequently, it was decided that the 1996 field experiment would be performed at the highest density experimented in the previous year (100 000 plants/ha or 12.5 cm between plants) and that plant stress would be implemented by contrasting two rates of nitrogen application. Row length was also shortened to 3.0 m to be

able to scout all the experimental plants in one day at each census. Second, slightly less resistant maize genotypes (A619Ht and A619Ht x bxbx) were used to generate intermediate aphid densities for which more data were needed.

In 1996, the field was sowed on May 24th as previously, but due to an epidemic of cutworms (Lepidoptera: Noctuidae) and to crows pulling the seedlings from the ground, *ca.* half the field had to be planted again the following weeks. Because of this, nitrogen application had to be modified. It was originally planned to do two applications, one at planting and one 4 weeks following emergence (both as side-dressing), but the second application had to be omitted to avoid the risk of burning the newly established seedlings. Hence, the two rates of nitrogen application actually implemented were equivalent to 35 kg of N/ha (low nitrogen) and to 70 kg of N/ha (high nitrogen). To ensure independence between treatments with respect to nitrogen regime, experimental plots were separated by strips of bare ground 3.5 m in width in all directions. Therefore, a split-plot design was not considered necessary for the 1996 experiment. Rather, a randomized block design was implemented with 4 blocks. Other details are as described previously.

### 5.2.3 Data collection

#### 5.2.3.1 Plant development

The aphid *R. maidis* initiates colonies on maize in the whorl, just before the tassel emerges (Foot, 1977). Crowding may be a factor involved in the natural decline of these colonies, but evidence shows that in maize this decline occurs as the tassel becomes exposed (Wright and Laing, 1980). Plants were thus monitored to establish the proportion of plants in a row with fully exposed tassels bearing immature flowers. The criterion used for excluding a plant with a fully exposed tassel was if visually $\geq 50$ % of the flowers were shedding pollen.

#### 5.2.3.2 Estimation of aphid numbers

The first census of aphids on the experimental plants was completed at the late whorl stage, just before the tassels begun to emerge. Because these first censuses always occurred while the tassels were still enclosed within the whorl leaves, it was not possible to count the aphids without severing the whorl. To minimize the number of plants destroyed in the experimental rows, only one plant was sampled in these, but aphids were also counted on one plant from the rows on each side of the experimental ones to obtain an average number of aphids for each experimental plot. On the following censuses, it was possible to estimate the

number of aphids on several plants within the experimental rows without damaging them. Classes with exponentially larger ranges of aphid numbers were establish to estimate the number of aphids. In 1994, these classes were: 1) no aphids, 2) 1-50, 3) 51-100, 4) 101-500, and 5) 500-1000. In 1995, the same classes were used, but class 2 was further subdivided into 2 classes (1-25 and 26-50) because several plants of the resistant inbred (CO273) obviously had a very small number of aphids. The mid value of a class was used to compute the mean number of aphids per plant. In 1996, the number of aphids could not be estimated using these classes since plants within a row were at different growth stages due to repeated sowing. Rather, a total of 3 plants were randomly sampled in the rows on each side of the experimental row at each census.

#### 5.2.3.3 Counts of ladybird eggs and pupae

Counts of ladybird eggs and pupae were made during a period encompassing the existence of *R. maidis* colonies in the late whorl and tasseling stages of the maize plants and a few days following the extinction of these colonies. Eggs were not counted in 1994 because pupae were found as soon as the second census, indicating that the initiation of the oviposition period was missed. Eggs and pupae were counted on all of the experimental plants (*ca.* 600 plants per census). Pupae were collected and brought to the laboratory to confirm species identification as adults. In 1994 and 1995, more than 95 % of the pupae were of

*Coleomegilla maculata lengi* Timberlake and the remaining were of *Propylea quatuordecimpunctata* L.. In 1996, the pupae were of *C. maculata lengi*, *P. quatuordecimpunctata*, *Hippodamia convergens* Guérin, *Adalia bipunctata* (L.), and *Harmonia axyridis* Pallas. Many pupae did not emerge in the lab so the accurate calculation of the proportions of the different species was not possible for 1996. However, 57 % were of *C. maculata lengi*, a percentage based on species identification in the field (the author is confident about this value because of his familiarity with *C. maculata*'s life stages).

To minimize intraguild predation, eggs and larvae of other aphid predators, mainly chrysopids, were removed at each census.

#### 5.2.3.4 Statistical analyses

The peak number of aphid per plant and the total number of pupae per plant were analyzed by ANOVA. The number of ladybird eggs and pupae per plant, and the survivorship of ladybirds (the ratio of pupae to eggs) were analyzed in relation to peak aphid number by non-linear regressions using the DUD method (=Doesn't Use Derivatives, also called the false position method) in the NLIN procedure of the Statistical Analysis System (SAS Institute, 1988, pp. 675-712).

## 5.3 Results

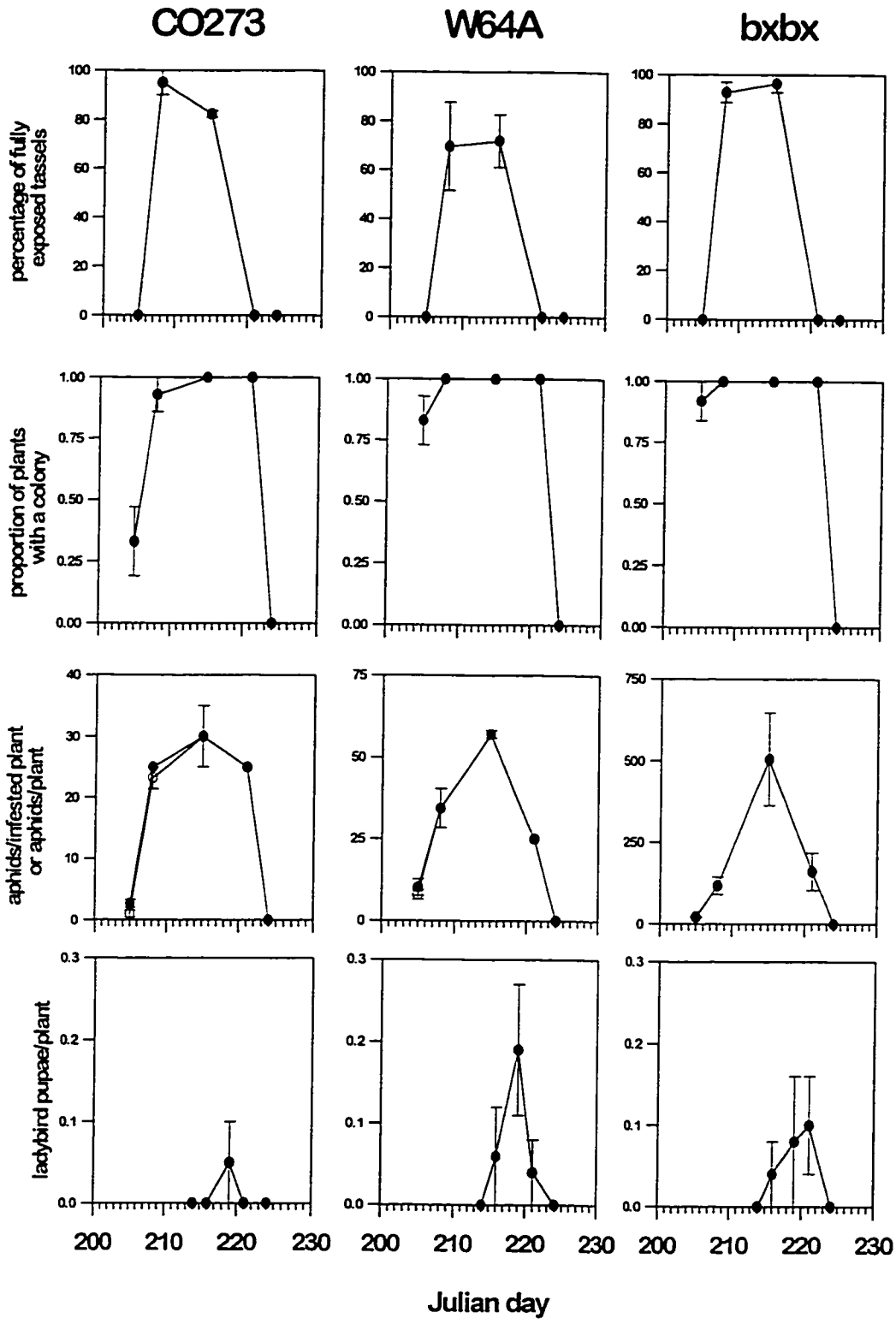
### 5.3.1 Dynamics at the different trophic levels of the system

The sequence of events at the maize plant, the aphid, and the ladybird beetle levels displayed an impressive constancy in this tritrophic system studied over three field seasons (Fig. 5.1a-g). Synchronism is evident between the maturation of the tassels and the rise and fall in the number of aphids per plant. Aphids increased while the tassels were about to emerge from the whorl or as soon as the tassels begun to emerge. They decreased as the tassels emerged and started to shed pollen. The tassels were no longer a resource available to the aphids when all the plants were at the pollinating stage.

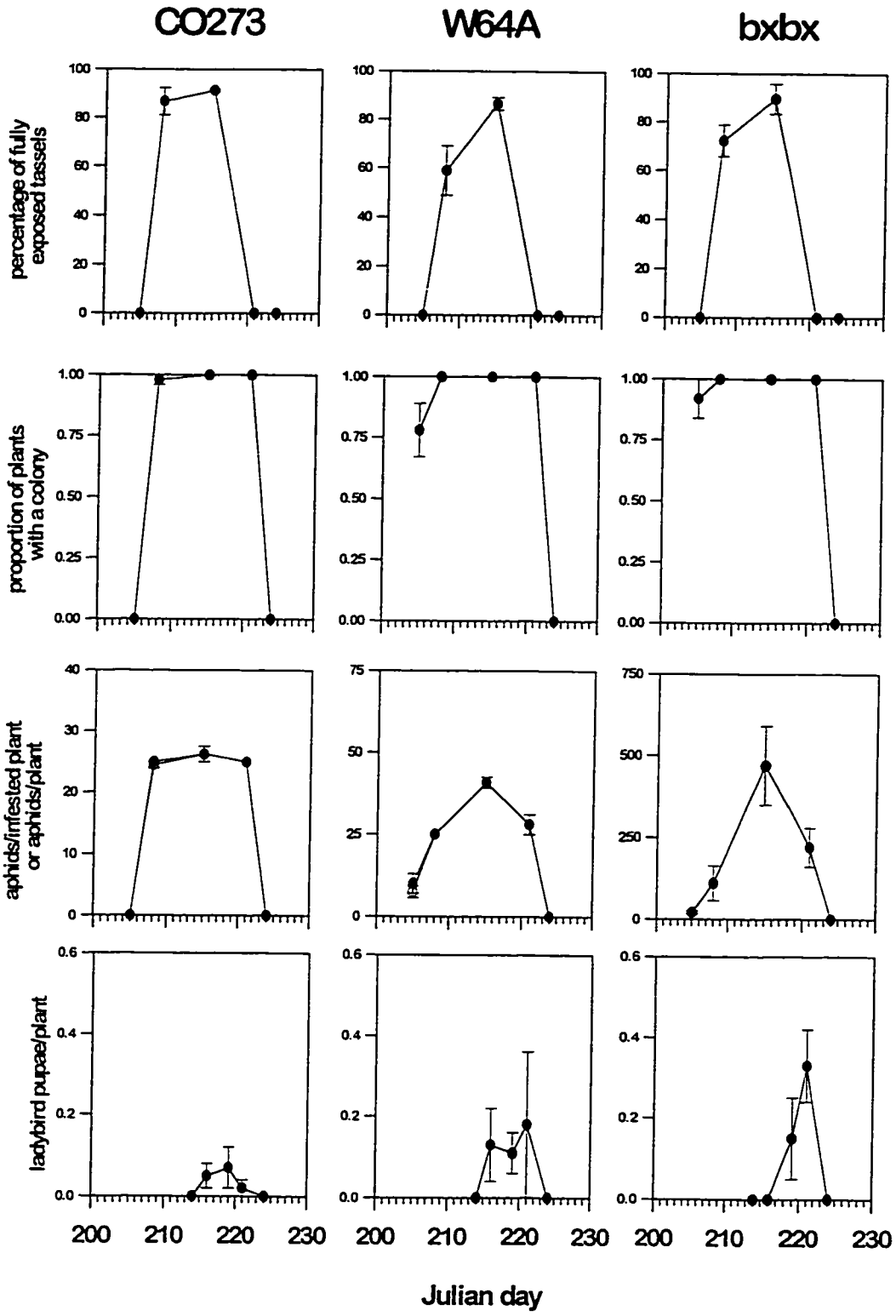
The dynamics of the aphid infestation in each year were however different with respect to the rates of increase and of decrease. These differences among years can be related to differences in temperature experienced by both the plant and the aphid. The growth rate of maize has been modeled as a quadratic function of temperature with an optimum at 30 °C (Major *et al.*, 1976) and Noda (1960) reported that the development time of *R. maidis* is fastest at 25 °C, but longer below and above this temperature. Average daily temperatures recorded on site help explain the role played by this extrinsic factor in mediating the interaction between the plant and the aphid or by directly acting on the insect.

Figure 5.1. Dynamics in the field of the tritrophic system composed of differentially resistant maize genotypes, the corn leaf aphid (*R. maidis*), and ladybird beetles. The inbreds CO273 (resistant), W64A (partially resistant), and bxbx (susceptible) were planted in 1994 and 1995. The inbreds A619Ht (resistant) and bxbx (susceptible) and the cross (F1s of A619Ht x bxbx; partially resistant) were planted in 1996. The percentage of plants with an exposed tassel (not shedding), the proportion of plants without an aphid colony, the number of aphids per plant (hollow circles) and the number of aphids per infested plant (filled circles), the number of ladybird eggs (1995 and 1996), and the number of ladybird pupae are shown in relation to Julian day. Each point represents the mean of 4 replicates with its standard error. A) 1994, low density (25 000 plants per ha or 42.5 cm between plants in a row). B) 1994, intermediate density (50 000 plants per ha or 20.0 cm between plants in a row). C) 1994, high density (100 000 plants per ha or 12.5 cm between plants in a row). D) 1995, low density. E) 1995, intermediate density. F) 1995, high density. G) 1996, high density (35 kg of N/ha). H) 1996, high density (70 kg/ha).

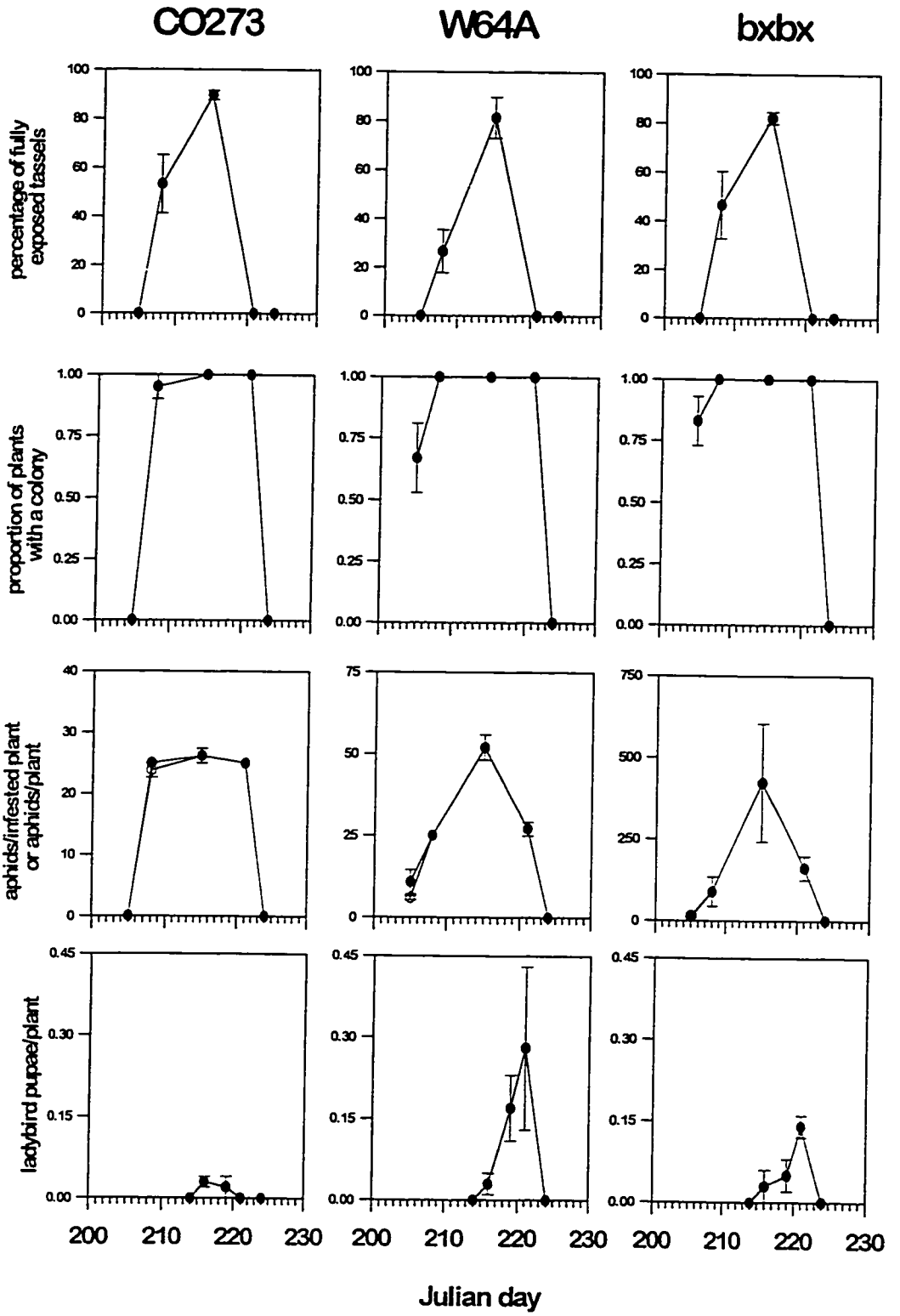
a) 1994, low density



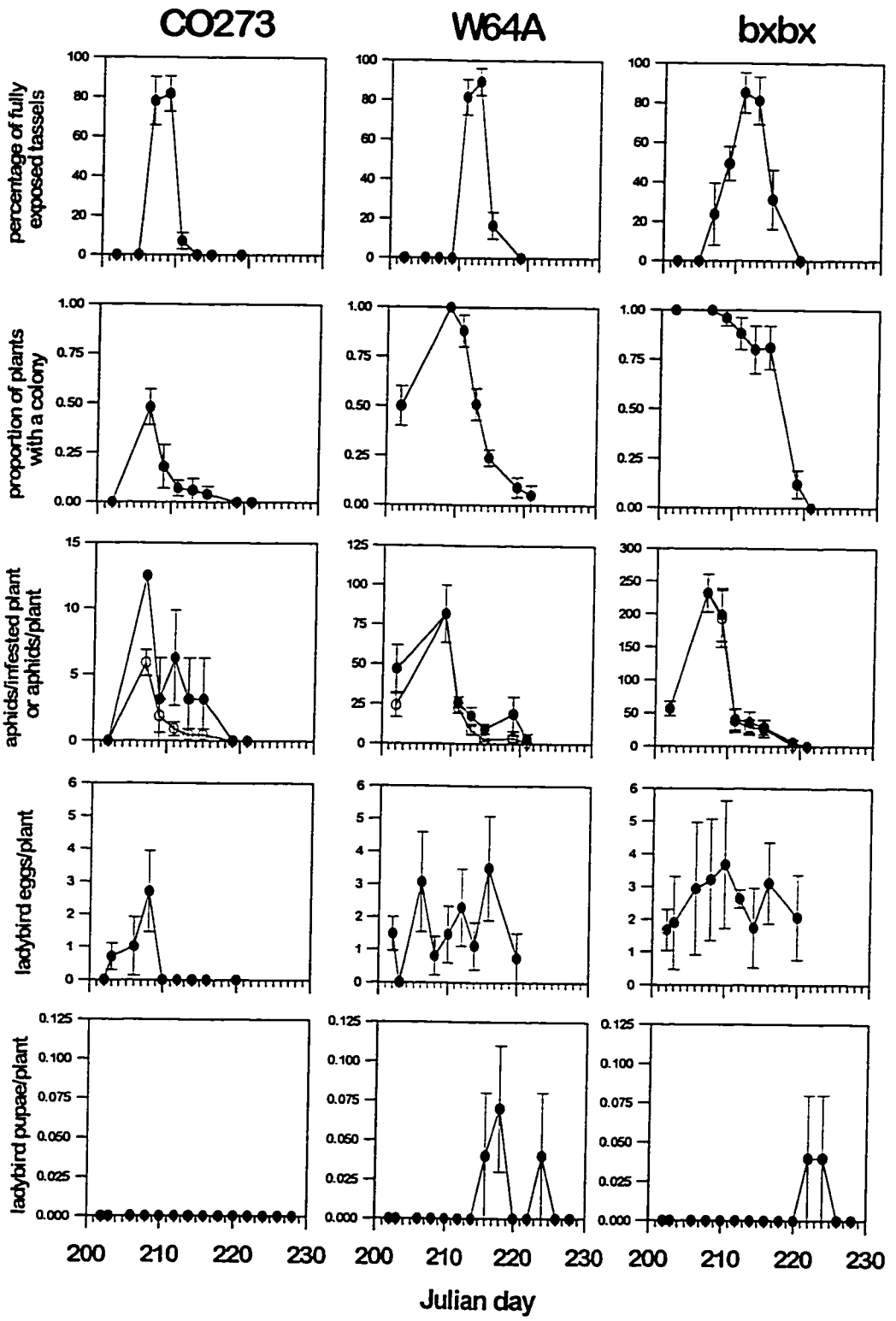
b) 1994, intermediate density



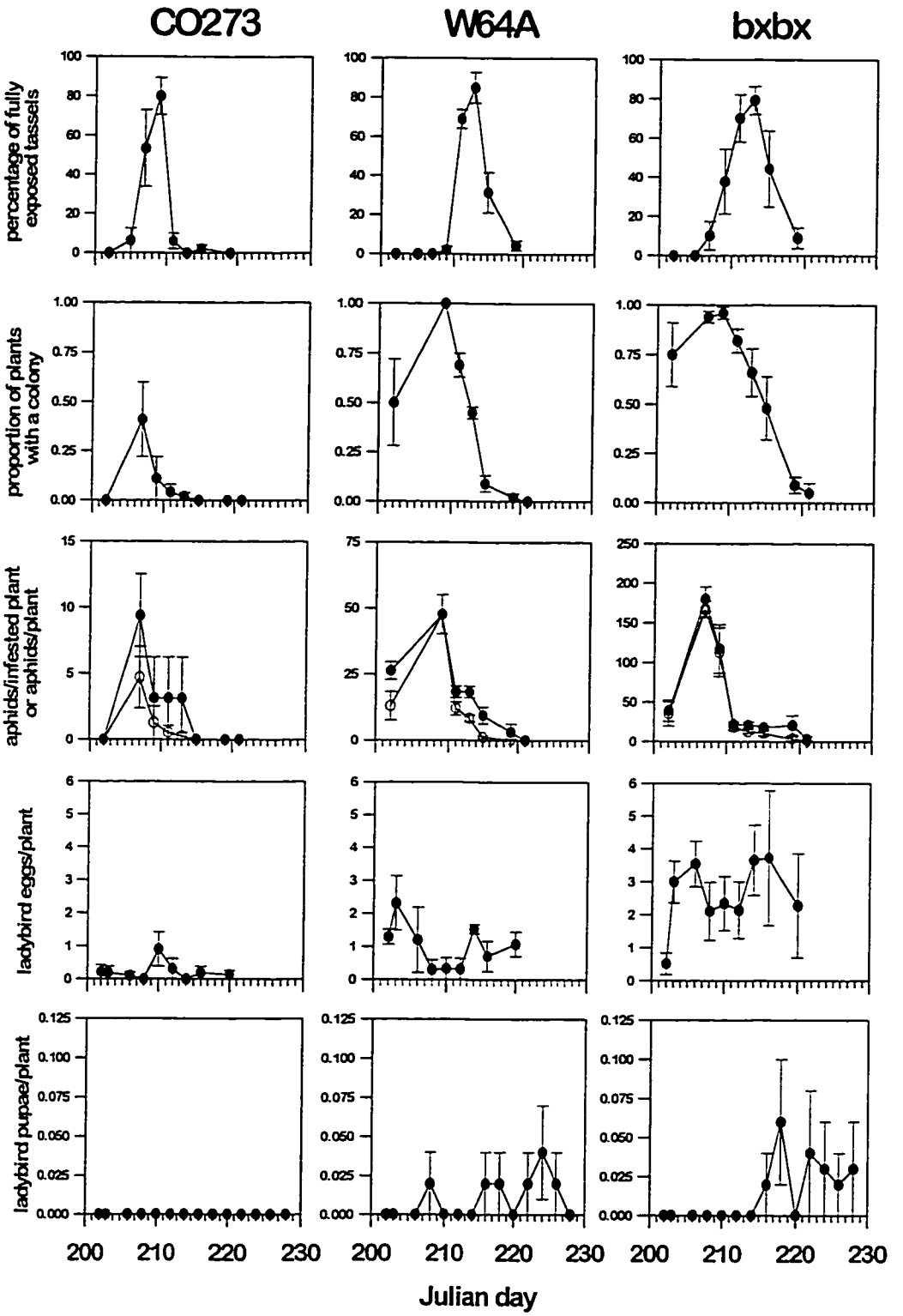
c) 1994, high density



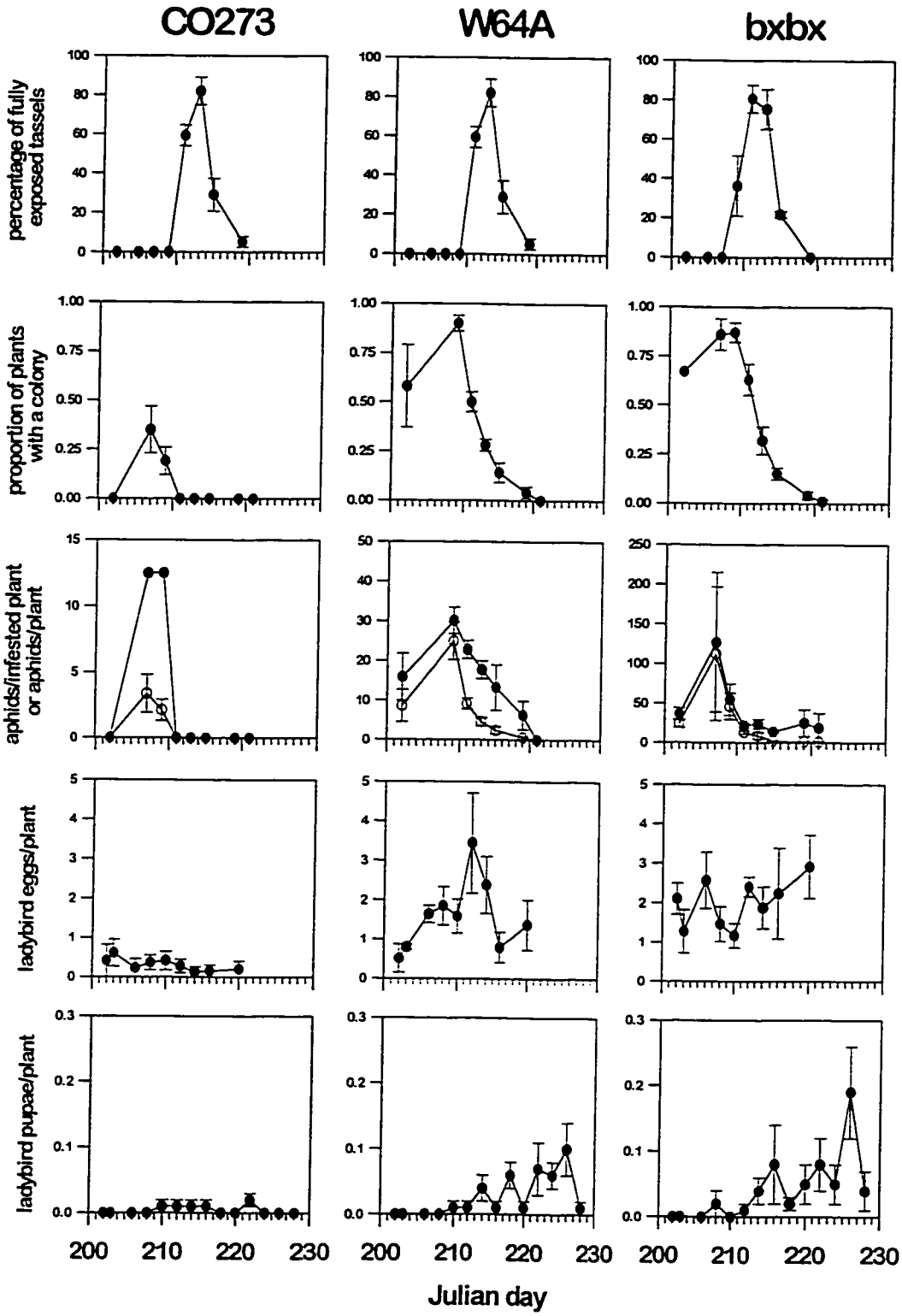
d) 1995, low density



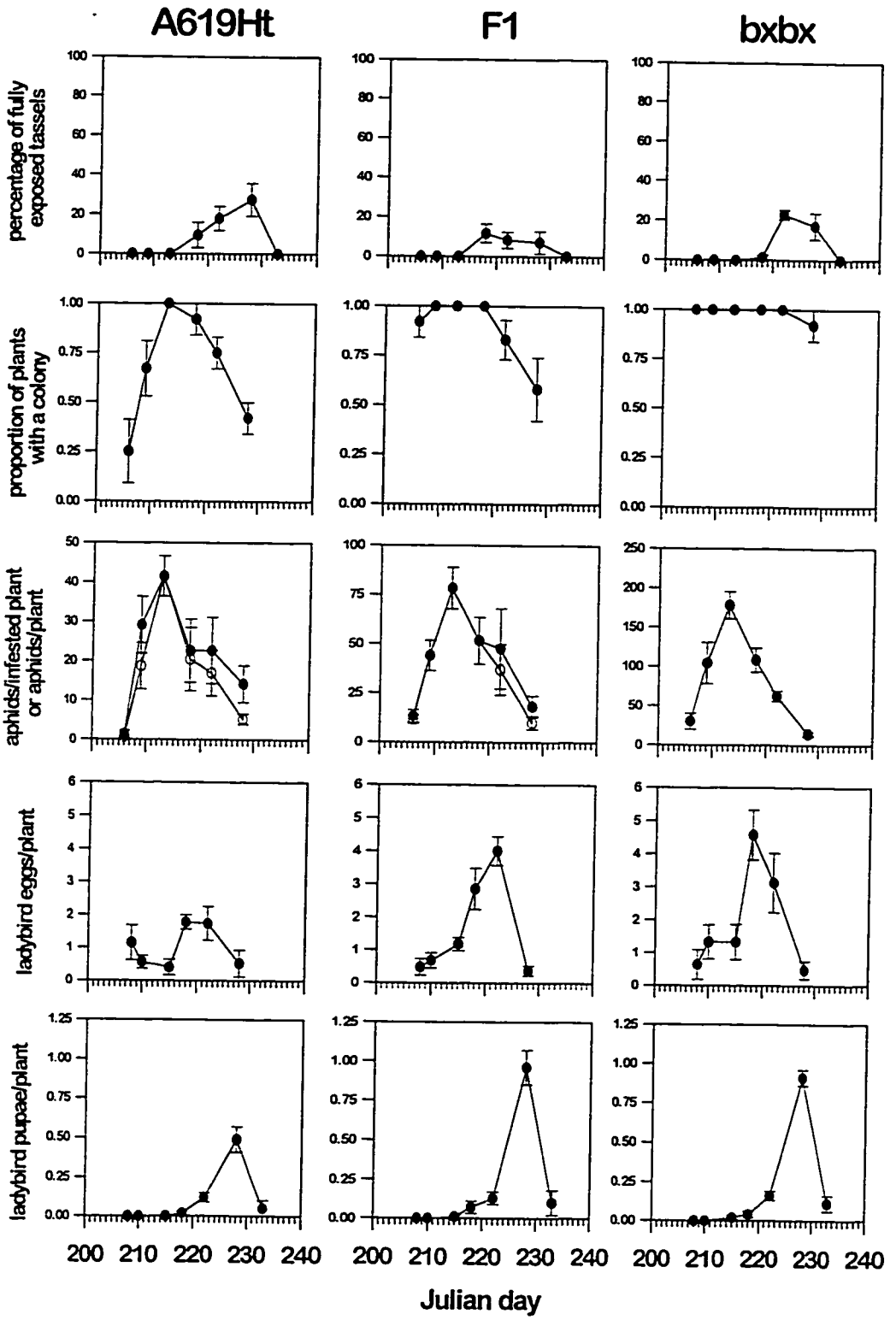
e) 1995, intermediate density



f) 1995, high density



g) 1996, low nitrogen



h) 1996, high nitrogen

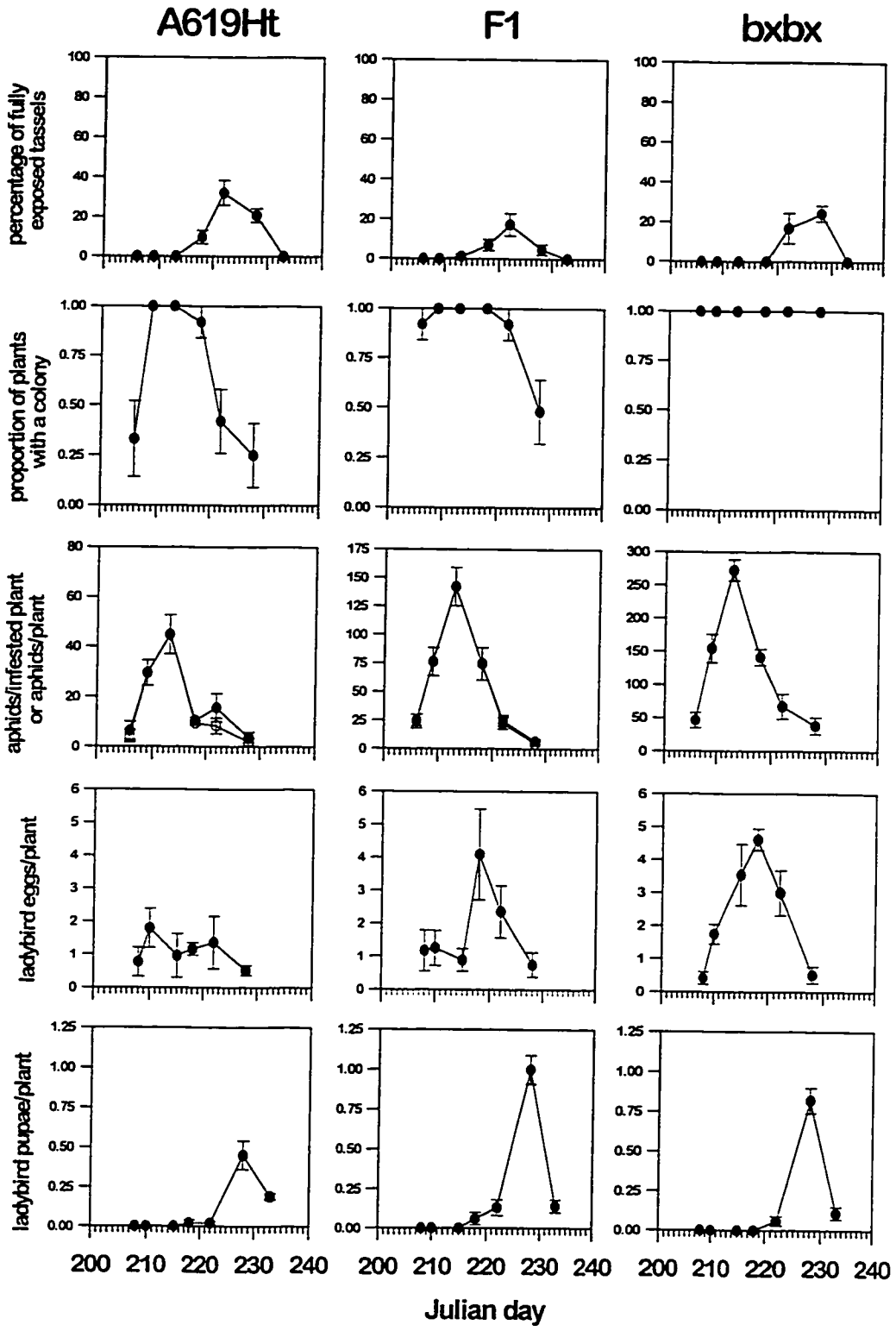
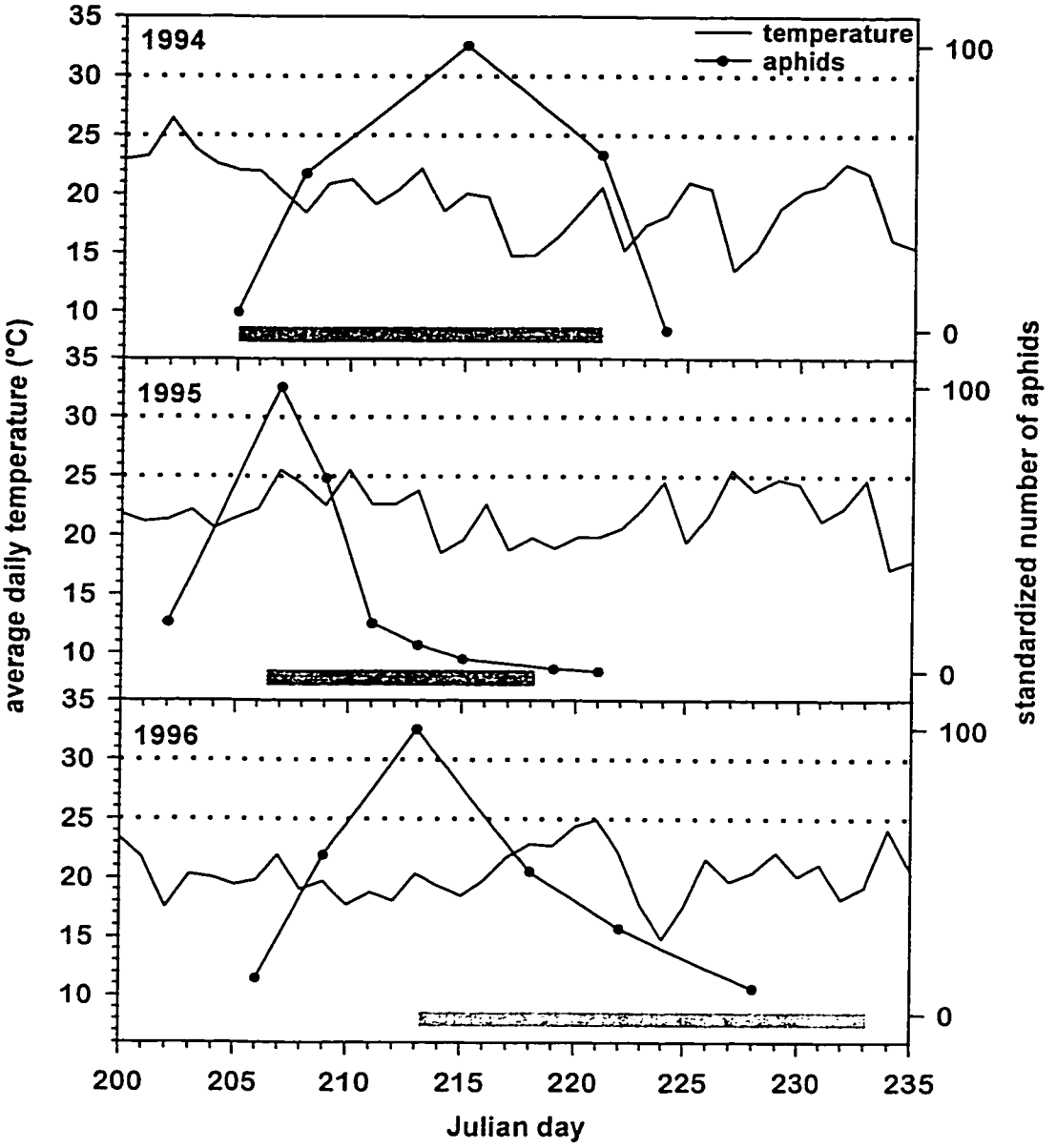


Figure 5.2 shows how deviations from these reference temperatures shape the rise and fall in aphid numbers. In 1994, as average daily temperatures dropped nearly constantly over the period aphids were present on the tassels, and well below 25 °C for the whole period, there was a concomitant slow development of the aphids. Also, temperatures being far from the optimum for maize, maturation of the tassel was delayed and in turn, these conditions widened the period over which aphids could use their resource. In 1995, average daily temperatures were closer to the optimum for both aphids and maize, with the consequence that the aphids increased rapidly while the tassel matured over a shorter period of time. In 1996, the increasing phase of aphid colonies occurred during a period where average daily temperatures were below 25 °C, while the declining phase experienced a sudden, but short, burst of temperature close to the optimum, hastening the decline. However, average daily temperatures were far from the optimum for maize and the tassels developed slowly. Year 1996 thus shared elements of both previous seasons in terms of temperatures and aphid dynamics responded to these elements.

Differences in temperatures among years also help in interpreting differences in the spatial distribution of aphid colonies (*see* Fig. 5.1a-g, proportions of plants with a colony). The cooler temperatures in 1994 not only allowed the aphids to exploit their resource for an extended period of time, but also favored infestation of all the plants available. Interestingly, plant

Figure 5.2. Average daily temperatures recorded at the Central Experimental Farm, Agriculture Canada, Ottawa, during aphid infestations in 1994, 1995, and 1996. The horizontal dotted lines depict the optimum temperatures for growth for maize (30 °C) and for the aphid *R. maidis* (25 °C). The shaded rectangles indicate the period of time tassels were exposed, averaged across treatments. Aphid numbers have first been standardized on a scale of 0-100 % using peak aphid number to compute deviations in each treatment and these standardized values were then averaged across treatments to yield the overall shape of the rise and fall in aphid numbers in a given year.


1994
1995
1996
temperature
aphids
average daily temperature (°C)
standardized number of aphids
Julian day
35
30
25
20
15
10
100
0
200
205
210
215
220
225
230
235

resistance did not influence this pattern of host utilization in 1994. In contrast, the warmer temperatures of 1995 reduced the time available to the aphids to exploit their host at the tasseling stage, and while aphids were able to colonize more plants as resistance decreased, they did so for a short period of time. In 1996, a season intermediate with respect to temperatures, elements of both previous seasons are apparent. While aphids were able to colonize all the plants available at all levels of resistance, they did so for a prolonged period of time as resistance decreased. These contrasting patterns observed between years strongly suggest that there is a strong interaction between temperature and host resistance in the spatial and temporal exploitation of the resource by the aphids.

Plant stress on the other hand, be it imposed by increasing plant density or by varying nitrogen rate, did not influence the patterns previously described (Fig. 5.1a-g). However, plant stress affected peak aphid number as it significantly decreased with increasing plant density (1994 and 1995: Table 5.1; Fig. 5.3a-b) and with low nitrogen application (1996: Table 5.2; Fig. 5.4).

Table 5.1. ANOVA on peak aphid number (data transformed to [$\log_{10}$ (number + 1] prior to analysis to stabilize variance) for the split-plots performed in the field in 1994 and 1995. Planting density was the factor in the main plots and the effect was tested against the proper error term (i.e. block*density).

| Source | *d.f.* | *MS* | *F-value* | *P* |
|---|---|---|---|---|
| Block | 3 | 0.0924 | 1.26 | 0.2988 |
| Plant density | 2 | 0.4767 | 18.06 | 0.0029 |
| Error (Density) | 6 | 0.0264 | | |
| Year | 1 | 2.8998 | 39.65 | <0.0001 |
| Inbred | 2 | 9.5528 | 130.63 | <0.0001 |
| Density*Year | 2 | 0.1557 | 2.13 | 0.1310 |
| Density*Inbred | 4 | 0.0836 | 1.14 | 0.3487 |
| Year*Inbred | 2 | 0.7149 | 9.78 | 0.0003 |
| Density*Year*Inbred | 4 | 0.0216 | 0.30 | 0.8795 |
| Error | 44 | 0.0731 | | |

Figure 5.3. Effect of host-plant resistance and planting density on peak aphid number in the field in (a) 1994 and (b) 1995. CO273: resistant inbred; W64A: partially resistant inbred; bxbx: susceptible inbred. Low density: 25 000 plants/ha or 42.5 cm between plants in a row. Intermediate density: 50 000/ha or 20.0 cm between plants. High density: 100 000/ha or 12.5 cm between plants. Means and standard errors are shown.

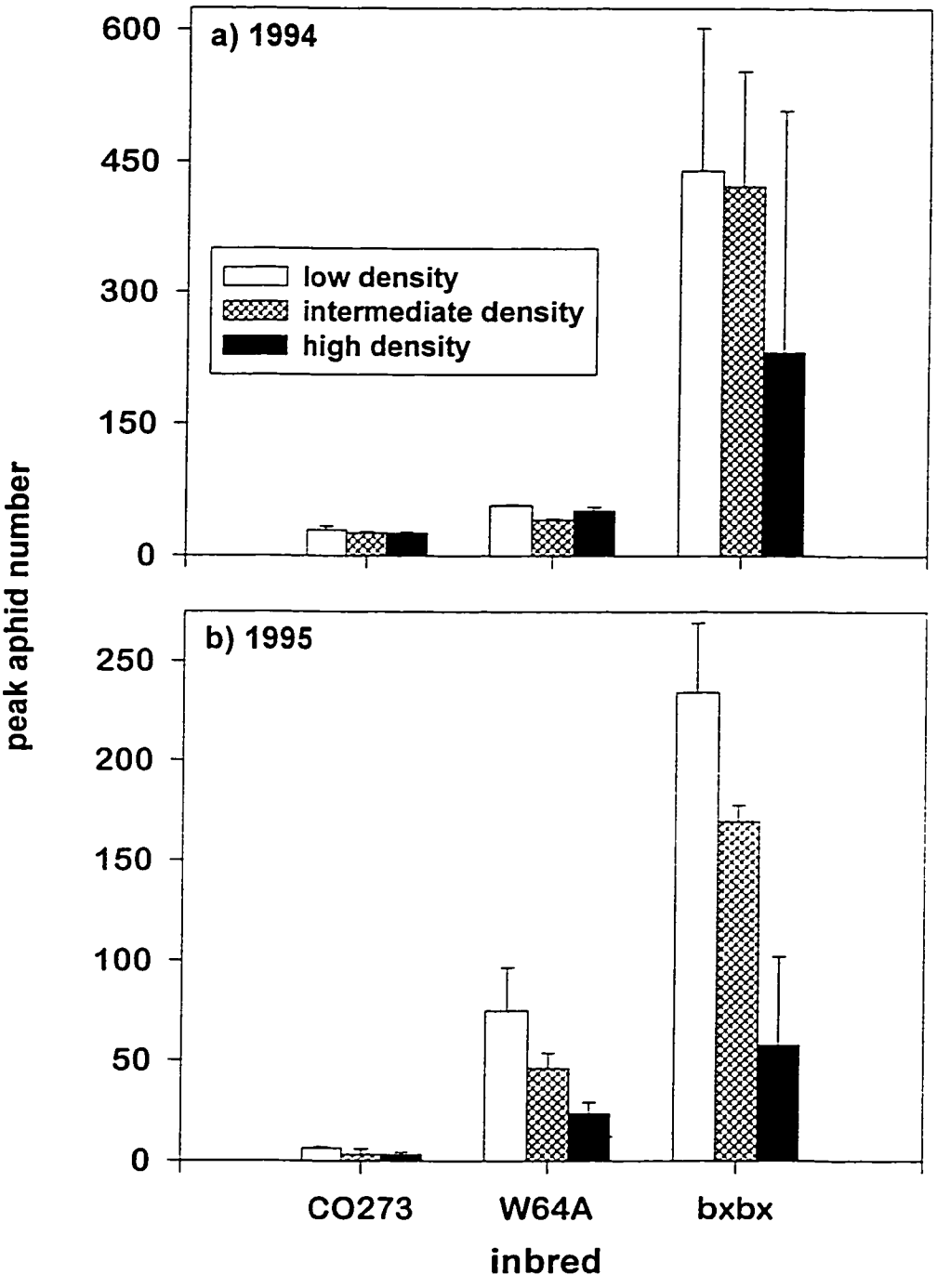
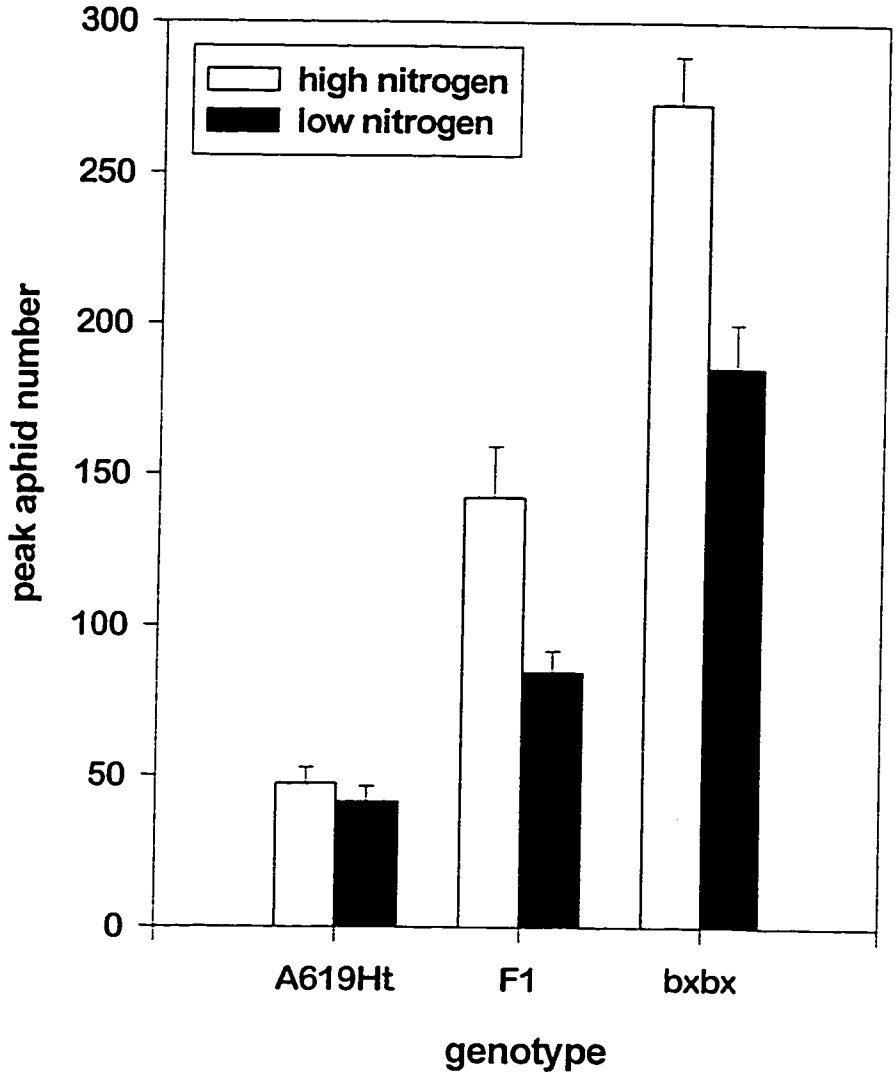

a) 1994
600
450
300
150
0
low density
intermediate density
high density
b) 1995
250
200
150
100
50
0
peak aphid number
CO273
W64A
bxbx
inbred

Table 5.2. ANOVA on peak aphid number for the 1996 experiment in which 2 rates of nitrogen application were performed (35 and 70 kg N/ha). Plant density was kept at 100 000 plants/ha.

| Source | *d.f.* | *MS* | *F-value* | *P* |
|---|---|---|---|---|
| Block | 3 | 1003.62 | 2.05 | 0.1498 |
| Genotype | 2 | 69539.39 | 142.16 | <0.0001 |
| Nitrogen | 1 | 15217.79 | 31.11 | <0.0001 |
| Genotype*Nitrogen | 2 | 3399.28 | 6.95 | 0.0073 |
| Error | 15 | 489.18 | | |

Figure 5.4. Effect of host-plant resistance and nitrogen level on peak aphid number in the field in 1996. Low nitrogen: 35 Kg N/ha. High nitrogen: 70 Kg N/ha. Plant density was kept at 100 000/ha. Means and standard errors are shown.


300
250
200
150
100
50
0
high nitrogen
low nitrogen
peak aphid number
A619Ht
F1
bxbx
genotype

In both types of experiment, plant stress resulted in more variation in the peak number of aphids as resistance decreased, though the interaction term in the ANOVA on the plant density experiment was not significant (Table 5.1). The reason for the absence of a significant interaction between maize genotype and plant density (Table 5.1) and its presence with nitrogen level (Table 5.2) is due to the logarithmic transformation operated on the 1994-95 data to stabilize the variance prior to the ANOVA. Due to this transformation, comparisons among treatments are made on a linear scale.

The differences observed in peak aphid number at different planting densities and nitrogen levels were mainly mediated by plant status since most of the ladybird eggs were laid after the number of aphids started to decline (Fig. 5.1d-h). Hence, the impact of ladybird larvae on peak aphid number is likely to have been negligible and independent of the level of plant stress or plant resistance. Therefore, peak aphid number can be used as an independent variable to analyze the reproductive response of ladybird beetles in relation to variations in prey availability and, in turn, to analyze how prey availability affects the survival of ladybird larvae.

### 5.3.2 The ladybird level

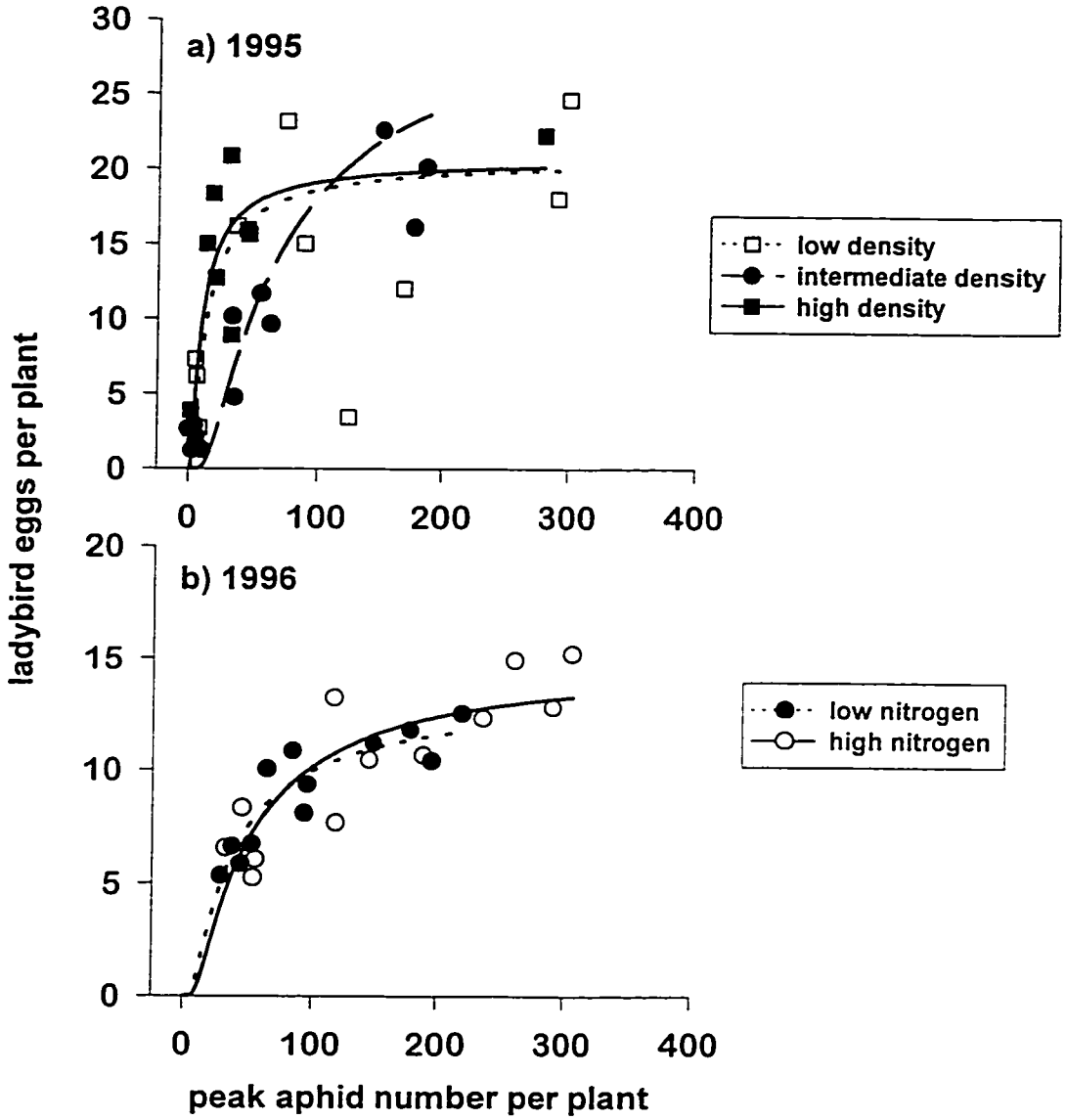
The number of ladybird eggs laid in relation to peak aphid number showed the typical saturating response (Fig. 5.5a-b). A non-linear equation of the form:

$$y = a^{*}e^{(-b/x)} \qquad (5.1)$$

was fitted to the data where $y$ is the total number of eggs laid by female beetles, $x$ is the peak number of aphids, $a$ is a parameter representing the asymptotically approached maximum number of eggs at saturation, and $b$ is a parameter representing the rate at which the value of $a$ is approached. In this equation, the larger the value of $a$, the larger the number of eggs at saturation, and the smaller the value of $b$, the faster the value of $a$ is approached. Independent regressions were performed for the different levels of plant density (1995) and nitrogen rate (1996). The values of parameters $a$ and $b$, given in the legend of figure 5.5a-b, can thus be compared among treatments, though the regression curves can be visually inspected.

The results show that female beetles expressed reproductive responses of similar shapes at low and high planting densities, rising very rapidly and saturating at the same level, while their response at intermediate planting density

Figure 5.5. Reproductive response of ladybird beetles in the field for (a) different planting densities and (b) nitrogen level. The equation $y = a*e^{(-b/x)}$ was fitted to the data. A) 1995, low density: a = 20.61, b = 10.35 ($R^2 = 0.44$, $F_{2, 10} = 20.43$, $p = 0.0003$); intermediate density: a = 31.66, b = 55.86 ($R^2 = 0.78$, $F_{2, 10} = 48.92$, $p < 0.0001$); high density: a = 20.64, b = 7.81 ($R^2 = 0.74$, $F_{2, 10} = 66.78$, $p < 0.0001$). B) 1996, low nitrogen: a = 13.38, b = 29.45 ($R^2 = 0.82$, $F_{2, 10} = 427.32$, $p < 0.0001$); high nitrogen: a = 15.08, b = 39.65 ($R^2 = 0.69$, $F_{2, 10} = 163.42$, $p < 0.0001$).


a) 1995
b) 1996
ladybird eggs per plant
peak aphid number per plant
low density
intermediate density
high density
low nitrogen
high nitrogen
0
5
10
15
20
25
30
0
100
200
300
400

was much slower and did not reach saturation within the range of aphids found on the plants (Fig. 5.5a). However, it should be emphasized that the scatter of data points around the regression curves decreases with increasing plant density, suggesting that, above and beyond the number of aphids present, distance between plants affected the reproductive response of the ladybird beetles.

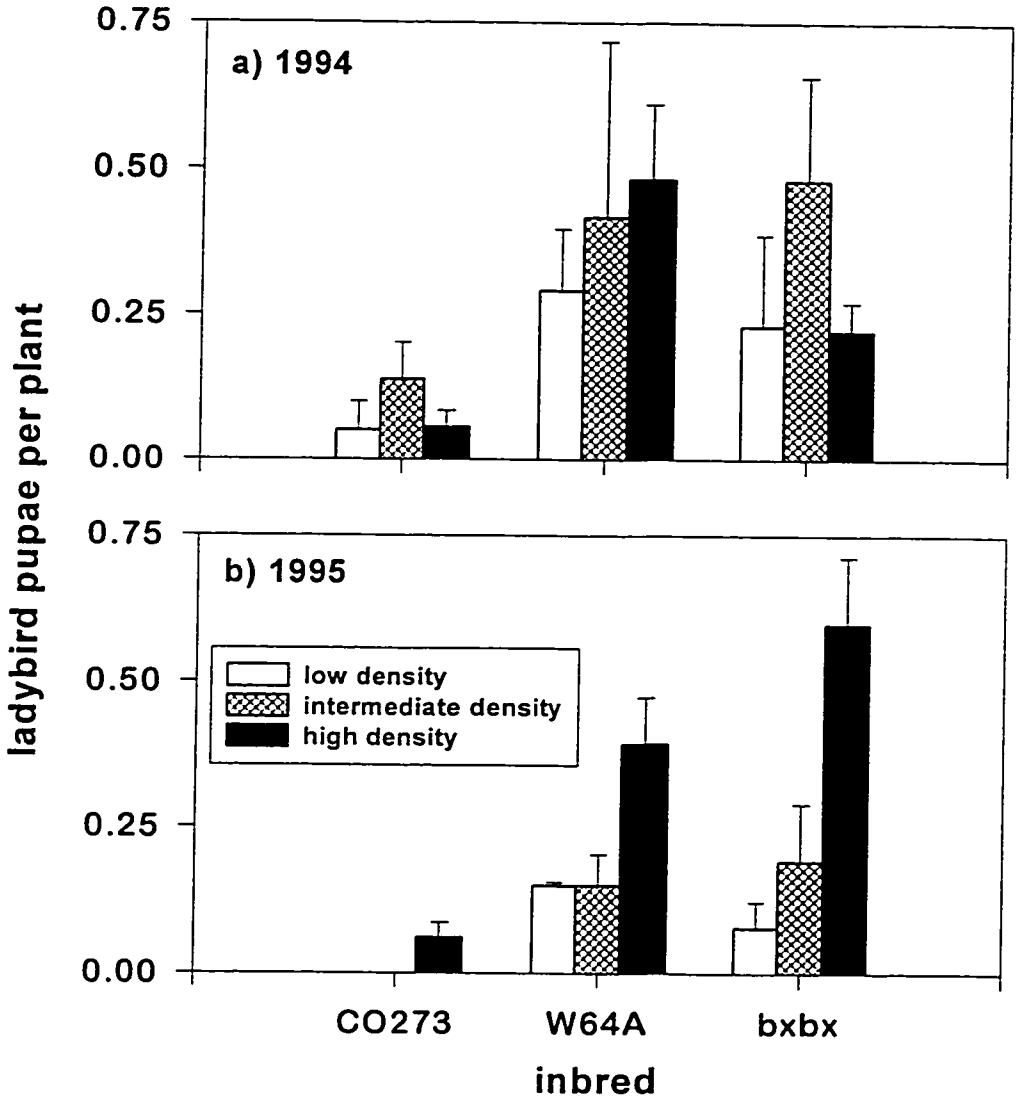
It is likely that plant density has mediated some interference in the evaluation made by female ladybird beetles as further evidenced by the reproductive response of the beetles in the 1996 experiment in which nitrogen level, rather than the distance between plants, was varied to stress the plants (Fig. 5.5b). While low nitrogen level resulted in lower number of aphids (Fig. 5.4), as increasing planting density did (Fig. 5.3), the shape of the reproductive response of the ladybird beetles in this treatment was practically identical to the one observed when the plants were supplemented with a higher rate of nitrogen.

Planting density also affected the total number of ladybird pupae per plant, but the effect of plant density depended on the year (Table 5.3; Fig. 5.6a-b). Despite that there were more aphids per plant at low and intermediate plant densities, less ladybird pupae were found in these treatments in 1995 compared to the high plant density treatment (Fig. 5.6b). In fact, none could be recovered in the plots planted at low and intermediate densities with the resistant inbred CO273 (Fig. 5.6b). This pattern was not observed in the 1994 experiment (Fig. 5.6a).

Table 5.3. ANOVA on number of ladybird pupae for the split-plots performed in the field in 1994 and 1995. Planting density was the factor in the main plots and the effect was tested against the proper error term (i.e. block*density).

| Source | *d.f.* | *MS* | *F-value* | *P* |
|---|---|---|---|---|
| Block | 3 | 0.200 | 7.30 | 0.0004 |
| Plant density | 2 | 0.173 | 3.71 | 0.0893 |
| Error (Density) | 6 | 0.047 | | |
| Year | 1 | 0.126 | 4.59 | 0.0378 |
| Inbred | 2 | 0.523 | 19.06 | 0.0001 |
| Density*Year | 2 | 0.167 | 6.09 | 0.0046 |
| Density*Inbred | 4 | 0.034 | 1.23 | 0.3137 |
| Year*Inbred | 2 | 0.035 | 1.28 | 0.2890 |
| Density*Year*Inbred | 4 | 0.053 | 1.93 | 0.1227 |
| Error | 44 | 0.027 | | |

Figure 5.6. Effect of host-plant resistance and planting density on the number of ladybird pupae in the field in (a) 1994 and (b) 1995. CO273: resistant inbred; W64A: partially resistant inbred; bxbx: susceptible inbred. Low density: 25 000 plants/ha or 42.5 cm between plants in a row. Intermediate density: 50 000/ha or 20.0 cm between plants. High density: 100 000/ha or 12.5 cm between plants. Means and standard errors are shown.


0.75
0.50
0.25
0.00
a) 1994
0.75
0.50
0.25
0.00
b) 1995
low density
intermediate density
high density
ladybird pupae per plant
CO273
W64A
bxbx
inbred

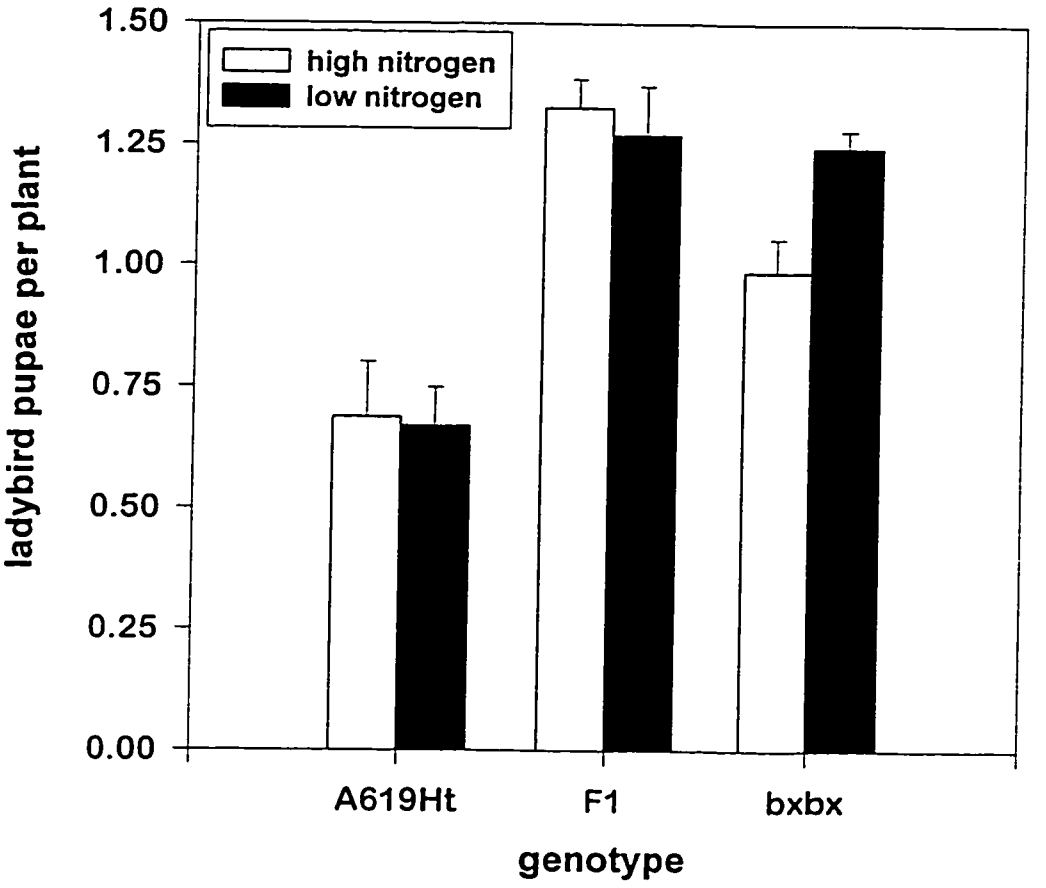
The major difference between the two years in which plant density experiments were performed lies in the spatial and temporal patterns of host exploitation by the aphids that have been described previously. In 1994, not only all the plants bore an aphid colony at one point, but they did so for an extended period of time, encompassing censuses during which pupae were found (Fig. 5.1a-c). In 1995, aphid colonies were observed on all the plants only in treatments including the partially resistant inbred W64A and the susceptible inbred bxbx, but the period of time where all the plants of these inbreds were infested was very short (Fig. 5.1d-f). These results provide evidence that the spatial distribution of aphid colonies affected foraging by ladybird larvae in the maize system. Hence, distance between plants is an important factor determining the foraging success of ladybird larvae, but less so as the distribution of aphid colonies is continuous from plant to plant.

With the 1996 nitrogen experiment, planned to remove the confounding effect of plant density on the effect of plant stress on ladybird beetles, the ANOVA failed to detect a significant contribution of this factor on the number of pupae found (Table 5.4; Fig. 5.7).

Table 5.4. ANOVA on the number of ladybird pupae for the 1996 experiment in which 2 rates of nitrogen application were performed (35 and 70 kg N/ha). Plant density was kept at 100 000 plants/ha.

| Source | *d.f.* | *MS* | *F-value* | *P* |
|---|---|---|---|---|
| Block | 3 | 0.023 | 0.88 | 0.4751 |
| Genotype | 2 | 0.807 | 30.30 | <0.0001 |
| Nitrogen | 1 | 0.022 | 0.84 | 0.3742 |
| Genotype*Nitrogen | 2 | 0.058 | 2.18 | 0.1473 |
| Error | 15 | 0.027 | | |

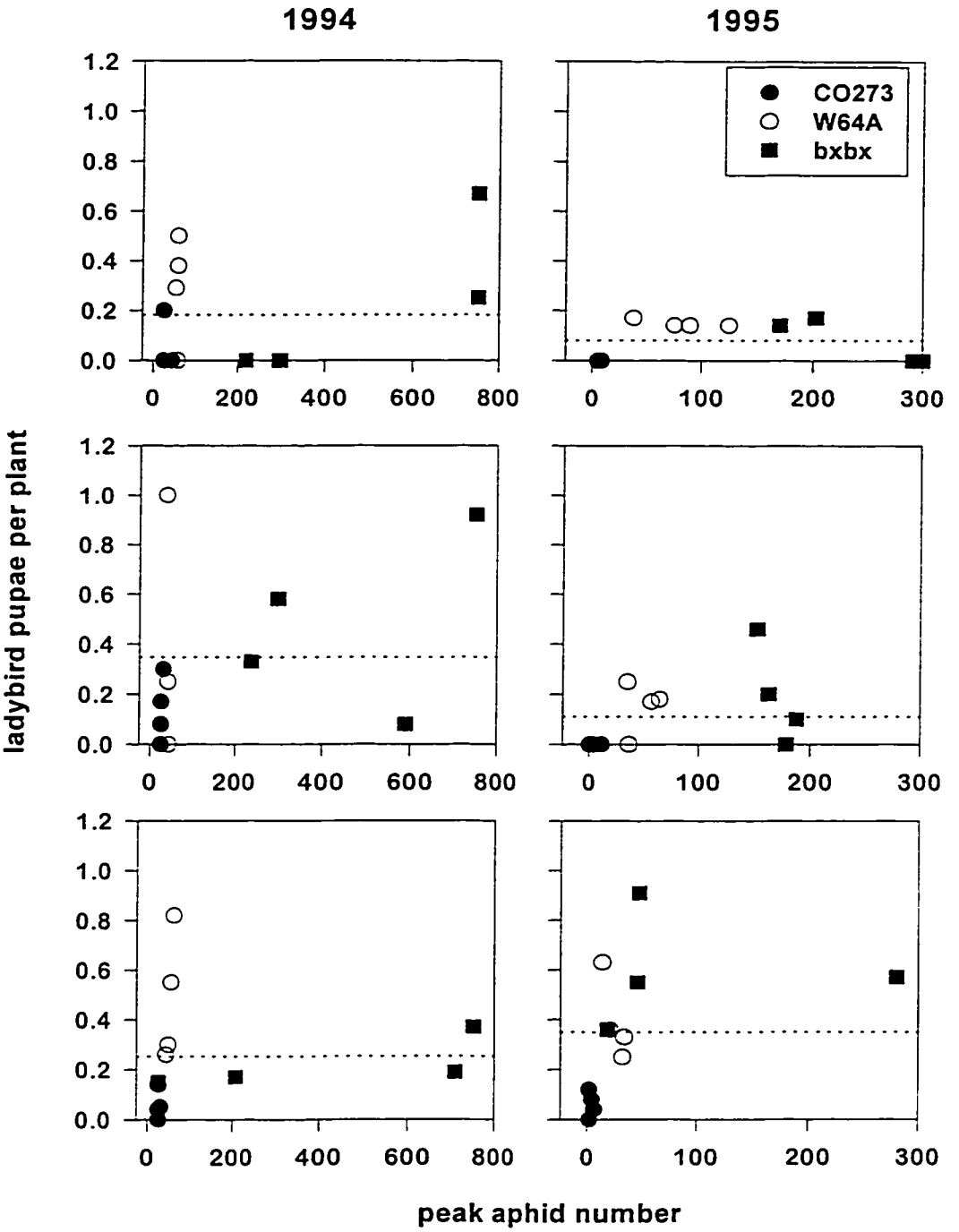
Figure 5.7. Effect of host-plant resistance and nitrogen level on the number of ladybird pupae in the field in 1996. Low nitrogen: 35 Kg N/ha. High nitrogen: 70 Kg N/ha. Plant density was kept at 100 000/ha. Means and standard errors are shown.


1.50
1.25
1.00
0.75
0.50
0.25
0.00
high nitrogen
low nitrogen
ladybird pupae per plant
A619Ht
F1
bxbx
genotype

Overall, ANOVAs indicated that the major source of variation at the ladybird level originated from maize genotype. This is somehow expected considering the wide range of aphid numbers generated by differential host resistance which spanned over two orders of magnitude in the present study. Plotting the total number of ladybird pupae against peak aphid number sheds light on the variability observed at the ladybird level. Also, by using data on a per replicate basis, it is possible to integrate the variability observed at the aphid level to obtain a finer grained picture of the variability at the ladybird level.

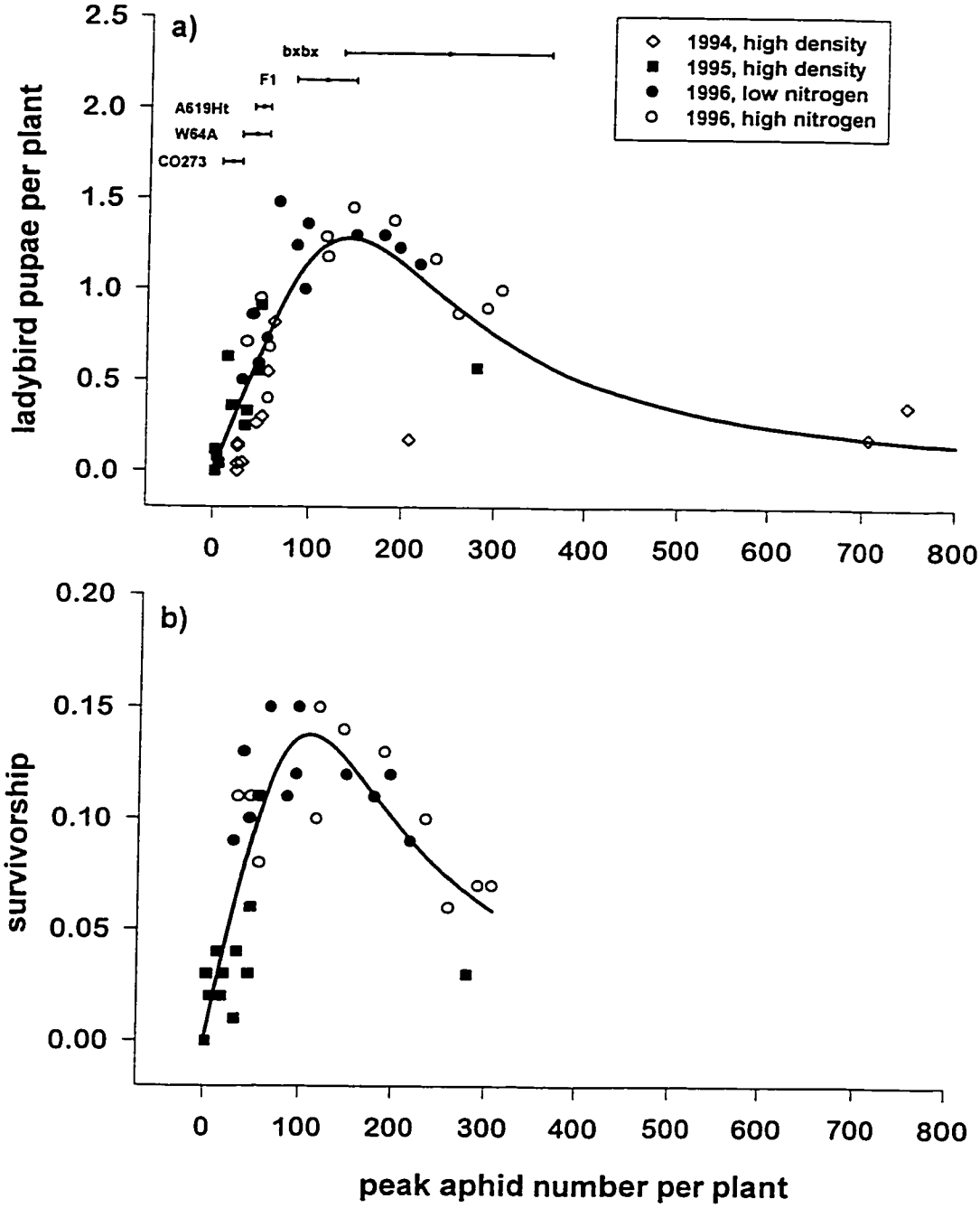
This has been done first with the data obtained in 1994 and 1995 (Fig. 5.8). What is striking in this figure is the very steep rise in the number of pupae recovered at low peak aphid numbers. Exceptions to this pattern are the low and intermediate plant density treatments performed in 1995, but an explanation for the low number of pupae recovered in these treatments has already been provided. Following this steep rise, the number of pupae recovered as peak aphid number increased do not seem to follow a clear pattern, though, if any, it would be to decrease. Since these data points are from the susceptible inbred bxbx, it may be argued that this pattern is related to some specific feature of this genotype. However, it is possible to dismiss this possibility since some of the data points from the bxbx inbred fall within the lower range of peak aphid numbers and fit perfectly the steep rise in the number of pupae recovered. The 1996 experiment included slightly less resistant genotypes specifically to help resolve the pattern from intermediate to high aphid numbers.

Figure 5.8. The total number of pupae in relation to peak aphid numbers in 1994 and 1995. Each point is a value for a different replicate. The horizontal lines are means across treatments. Top panel: low plant density (25 000/ha or 42.5 cm between plants). Middle panel: intermediate plant density (50 000/ha or 20 cm between plants). Bottom panel: high plant density (100 000/ha or 12.5 cm between plants).


1994
1995
CO273
W64A
bxbx
ladybird pupae per plant
peak aphid number
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
200
400
600
800
100
300

In figure 5.9a, all the data collected at the highest plant density tested across years were plotted on the same graph. The number of pupae recovered at the lower range of peak aphid number in 1996 fall on the same steep rise as the data points did in the previous years, confirming the pattern shown in figure 5.8. The 1996 experiment also clearly shows that, following this steep rise, the number of pupae declines as the peak number of aphid increases, as suspected from the data collected in the previous years. To determine whether the steep rise in the number of pupae recovered was the result of the steep rise in the number of ladybird eggs laid in the lower range of peak aphid number (Fig. 5.5a-b), the ratio of pupae to eggs was computed to yield an estimate of the survivorship of the immature stages of the ladybirds. The null hypothesis here would be that the steep rise in the number of pupae recovered would be the reflection of the increasing number of eggs laid in the lower range of peak aphid number, generating a straight horizontal line for the survivorship curve within that range. Figure 5.9b ( *note that data on ladybirds eggs are not available for 1994*) shows that this is not the case. The survivorship curve is even steeper within that lower range, suggesting that increasing peak aphid number is increasingly beneficial for the survival of ladybird larvae. There is a point, however, where the survivorship of ladybird larvae decreases as aphids increase in number.

Figure 5.9. (a) The total number of pupae and (b) the survivorship of ladybird larvae (the ratio of pupae to eggs) as a function of peak aphid number (PKA) for treatments at high plant density. The following equation was fitted to the data: $y = a*PKA / (1+(b*PKA)^c)$. The equation explained 69 % of the variation in the number of pupae ($F_{3, 45} = 134.2$, $p < 0.0001$) and 66 % of the variation in survivorship ($F_{3, 33} = 133.9$, $p < 0.0001$). The horizontal lines represent the 95 % confidence interval of peak aphid number computed for each genotype across treatments.


a)
b)
bxbx
F1
A619Ht
W64A
CO273
1994, high density
1995, high density
1996, low nitrogen
1996, high nitrogen
ladybird pupae per plant
survivorship
peak aphid number per plant
0.0
0.5
1.0
1.5
2.0
2.5
0.00
0.05
0.10
0.15
0.20
0
100
200
300
400
500
600
700
800

To link further the variability at the ladybird level to the variability at the aphid level, the 95 % confidence intervals in which the peak aphid numbers generated by the different maize genotypes lie have been computed on the pooled data across years for the highest plant density tested (Fig. 5.9a). The pattern obtained confirms the previous observation that variability at the aphid level decreases with increased resistance. However, it is at the lower range of aphid number that the variability at the ladybird level is the greatest. At the other extreme, plants lacking resistance generate large variations in the number of aphids.

## 5.4 Discussion

The results of this study exemplify some of the key elements of a theoretical model proposed by Hunter and Price (1992) in which heterogeneity at the plant level, be it intrinsic (e.g. resistance to herbivores, plant density) or extrinsic (e.g. nitrogen, temperature), induces heterogeneity at higher trophic levels. Here, variations in host-plant resistance allowed peak aphid number to extend over two order of magnitudes (Figs. 5.3 and 5.4), a range, to my knowledge, rarely achieved in other studies of tritrophic interactions. Also, increased resistance generally led to fewer plants to be exploited, or to be exploited for a shorter period of time (Fig. 5.1d-h). Plant stress, imposed by increasing plant density (in 1995) or by varying nitrogen rate (1996), led to a decrease in peak aphid number. This decrease induced by plant stress was magnified as resistance to the aphid decreased, resulting in more variation in peak aphid number. This interaction between plant stress and resistance on the aphid *R. maidis* has been previously observed in an experiment conducted in a growth chamber (Chapter II). Differences in temperature within and between growing season introduced more heterogeneity in the maize system (Fig. 5.2). Cooler temperatures extended the period during which the tassel was available as a resource for the aphid, and allowed for more plants to be colonized, overriding the effect of increased resistance on the spatial and temporal distribution of aphid colonies (e.g. Fig. 5.1a-c). Warmer temperatures had the opposite effect.

Heterogeneity at the plant level mediated variation at the ladybird level through the spatial and temporal distribution of aphid colonies and through the number of aphids. The reproductive response of ladybird beetles to peak aphid number was tighter as plant density increased (1995; Fig. 5.5a). This is particularly true for the response from intermediate to high peak aphid number. The better fit of the equation describing the ladybird reproductive response (equation 5.1) to the high plant density treatment in 1995 was reproduced in the 1996 experiment in which plant density was maintained constant at that level (Fig. 5.5b). In 1996, high nitrogen rate resulted in more aphids than in the low rate treatment, and ladybird beetles laid more eggs accordingly. The overall reproductive responses of ladybird beetles in both treatments were, however, very similar. These results suggest that distance between plants affected the oviposition response of female ladybirds. This may come about because distance between plants interferes with the aggregative response of ladybird beetles to aphid density. Kareiva (1987) conducted an experiment in which patches of goldenrod bearing aphid colonies were either continuous or separated by patches of mown grass. His results indicated that adult ladybird beetles expressed their aggregative response only when patches of goldenrod were continuous.

The heterogeneity at the aphid level generated by the plant level generated heterogeneity in the reproductive response of ladybird beetles (Fig. 5.5a-b). They

laid increasingly more eggs as peak aphid number increased, but from intermediate to high aphid number, their response tended to saturate. The points of inflection of the curves derived from equation 5.1 for the high plant density treatments (1995 and 1996) are approximately located between 50-100 aphids per plant. Interestingly, ladybird larvae survivorship started to decline just past this range (Fig. 5.9b). Below this range, and in absolute term, absolute peak aphid number was less subject to variation, but because of the steep rise in the reproductive response of ladybird beetles, small scale heterogeneity at the plant level can permeate and generate wide variations in the number of eggs laid by ladybird beetles. Above this range, peak aphid number was subject to more variation, but because the reproductive response of ladybird beetles tended to saturate at higher peak aphid number, heterogeneity brought about from the plant level has less impact on this response.

The tendency for the reproductive response to saturate when aphids are abundant has been related to satiation in ladybird beetles (Kareiva and Odell, 1987). Consequently, further increase in the number of aphids past the point where ladybirds females are satiated does not lead to a concomitant large increase in the number of eggs laid (Mills, 1982). According to the data collected here (Fig. 5.9b), however, the survivorship of the new generation of ladybird beetles both decreases on each side of the range of peak aphid number where the inflection

point is indicated in the reproductive response curves (Fig. 5.5). Again, heterogeneity at the plant level induced variation two levels above.

The decrease in the survivorship of the new generation of ladybird beetles below the range at which the reproductive response starts to saturate is as, phenomenologically speaking, ladybird females always overshoot. However, they do so less as peak aphid number increases in that lower range since their reproductive response was inversely density-dependent. This can be deduced from the properties of equation 5.1 for which the number of ladybird eggs is a decelerating function of peak aphid number. This means that there is more aphids per capita as peak aphid number increases, leading to increased survivorship within the lower range of peak aphid number.

The original hypothesis predicted that the survivorship of ladybird larvae would level-off as peak aphid number further increases. This prediction was based on the saturating reproductive response generally observed in predators (Holling, 1961) and which has already been given empirical support in ladybird beetles (Mills, 1982). This prediction is invalidated here since it was observed that survivorship of the new generation of ladybird beetles decreased with further increase in peak aphid number (Fig. 5.9b). This was, admittedly, an unexpected outcome. It is as if a scramble for resources operated, despite the fact that more aphids were available on a per capita basis. This sometimes occurs in ladybird

beetles (Kindlmann and Dixon, 1993), but scramble competition among larvae arises because eggs have been laid before the decline in the number of aphids and the removal of each aphid by predation early in the existence of a colony actually represents several aphids which would have been available a few days later. A tentative explanation for the apparent scramble competition in this study may stem from the developmental response (Murdoch, 1971) of ladybird larvae, i.e. the increasing demand for food as a predator grows in size. In the maize system, this developmental response is superimposed on the natural decline of the number of aphids with the maturation of the tassel since ladybird eggs are laid mainly when the peak number of aphids has been reached. This means that, as more ladybird larvae are present in the system, more face the threat of food shortage when aphid colonies on the tassels are on the verge of extinction. This can very well lead to the decrease in the survivorship of the new generation of ladybird beetles reported in the present study, yet this explanation is only partial since it does not provide an answer to the question of why the survivorship decreases rapidly. The following chapter presents the results of an experiment conducted to determine the survivorship curve of immature ladybirds for a range of initial number of larvae.

By focusing on the survivorship of a predator, the present study showed that heterogeneity at the plant level can generate heterogeneity up to the third trophic level. The survivorship of immature ladybirds varies greatly in the maize agro-ecosystem, but a small window exists in which the number of aphids,

mediated by host-plant resistance, can lead to stability in the whole system. In this sense, proponents of partial host-resistance will find support in favor of their argument. However, the results presented here indicate that intermediate resistance has little meaning if one does not know how insect pest density affects biological control agents. Six differentially resistant maize genotypes have been tested in the present study. Hence, 4 of them were partially resistant to the aphid *R. maidis*. Only one, however, generated the range of aphid numbers that approximately matched the range that stabilized the survivorship of immature ladybirds (A619Ht x bxbx; Fig. 5.9). The present study also points out that ladybird beetles cannot be considered as potential biological control agents against *R. maidis* in the maize agro-ecosystem. The temporal refuge afforded by the whorl leaves enclosing the tassels of maize plants delays the onset of the reproductive numerical response of the ladybird beetles beyond the point where the aphids have already expressed their full potential in terms of number. But more importantly, the results presented here suggest that the number of aphids needed to sustain immature ladybirds is high. However, the use of partial resistance to set an upper ceiling to the number of aphids present in the system, and in turn, attract ladybird beetles, may be an avenue to explore as these predators may prey on other insect herbivores that feed on maize (Evans and England, 1996).

— Chapter VI —

# CANNIBALISM IN MIXED-AGE AND EVEN-AGED POPULATIONS OF *COLEOMEGILLA MACULATA LENGI* TIMBERLAKE (COLEOPTERA: COCCINELLIDAE) LARVAE

## 6.1 Introduction

Cannibalism is an extreme form of direct interference among individuals of a population (Crowley *et al.*, 1987). By eliminating a potential competitor, the cannibal increases its share of food resources. This behavior might be especially useful when abundance of resources changes rapidly with time. Ladybird larvae face exactly this situation when they feed on aphids. Aphid colonies typically increase and then decrease in size over a short period of time, even in the absence of predation (Dixon, 1985). This dynamic in the prey population causes a decrease in the per capita prey availability as the per capita requirement for food increases in the predator population because ladybird larvae are reaching larger sizes. Hence, female ladybirds must decide when and how many eggs they should lay. These questions have been recently addressed in the literature as a problem of optimization. Hemptinne *et al.* (1992) suggested that when confronted with the short duration of an aphid colony, female ladybirds must lay their eggs early in its development because laying too late may keep the larvae from completing their development before the colony goes extinct.

Kindlmann and Dixon (1993) have developed a formal model of how many eggs female ladybirds should lay to maximize their reproductive output, assuming that they also optimize for time. Their model indicates that when a certain threshold initial density of eggs is reached, there is a sharp decline in the final ladybird biomass attained because of increased competition for prey and the concomitant increase in the rate of cannibalism. Hence, there is an optimal number of eggs that maximizes the reproductive output of female ladybirds.

By laying their eggs in a narrow window of time, as optimal theory predicts, female ladybirds generate even-aged populations of larvae. This strategy ought to minimize cannibalism among larvae since this particular form of interference competition is more likely to occur between individuals with different relative body sizes (Polis, 1981; Fox, 1975). Consequently, the present study examines the importance of cannibalism in even-aged populations of *Coleomegilla maculata lengi* larvae by contrasting the outcomes with the fates of mixed-age populations, i.e. populations that are the product of a constant rate of oviposition. Because the rate of cannibalism probably depends on the likelihood of encounters between larvae (Polis, 1981), a component of density-dependence, such comparison between the two types of populations cannot discriminate between effects due on the one hand to density and on the other hand to the behavioral propensity to cannibalize. Since I was interested in the latter, I minimized the effects of varying encounter rates by using small arenas that were effectively

searched by young larvae within a period of 24 hours. Also, since cannibalism is dependent on the level of alternative food resources (Polis, 1981; Fox, 1975), I minimized this effect by providing the larvae in these laboratory populations with *ad libitum* artificial diet.

Under the conditions implemented in this experiment, the results reported here can be considered as the maximum level of interference resulting in the death of a potential competitor that can be derived for both mixed-age and even-aged populations of *C. maculata* larvae when food is not limiting and when encounter rate is not a factor. I hypothesized that cannibalism would be present in both types of population, although it would be higher in mixed-age populations.

## 6.2 Material and methods

The *C. maculata* for this study were from a laboratory culture. One-day old larvae from eggs of 15 different females were randomly assigned to the different treatments. One-day old larvae were used in this experiment since I was interested in measuring the extent of cannibalism after dispersal from the egg cluster. Sibling egg-cannibalism has been shown not to depend on cluster size in the laboratory in *Coccinella septempunctata* L. (Dixon and Guo, 1993) and in the field in *Harmonia axyridis* Pallas (Osawa, 1993).

The larvae were placed in 75 ml vials according to the schedule in Table 6.1. Preliminary observations indicated that these vials were small enough for a young larva to search completely within 24 hours. The larvae in the present experiment received a modified dry artificial diet composed of 50 % of a diet originally developed by Rogers *et al.*, (1972) for *Propylea quatuordecimpunctata* L. (50 % yeast-free desiccated beef liver [Swiss Herbal Remedies Ltd, Markham, Ontario], 40 % brewer's yeast [no origin specified] , and 10 % sucrose [Fisher Scientific] [w/w]) and of 50 % of wildflower pollen available from health stores (Bourgel-Kamouraska Natural Bee Pollen, Natrum Inc., St-Joseph-du-Lac, Québec). C. maculata can complete its whole life cycle on this diet with larval mortality being rare. The diet was renewed daily. An eppendorf filled with distilled water and with a cotton ball fitted at the opening was provided in each vial.

Mixed-age populations were generated by staggering introductions over a period of 13 days (2, 4, 5, and 7 larvae total) or by introducing 1, 2, 3, or 4 larvae per day over the same period (13, 26, 39, and 52 larvae total). A period of 13 days represents the average larval duration for individual *C. maculata* reared under the conditions this experiment was performed (25 °C, 60% R.H., 16L:8D). Even-aged populations were generated by introducing all larvae at once. The treatment levels for these populations were 2, 5, 13, and 39 larvae total. Controls consisted of vials with only one larva. Three replicates were performed for controls and each population level. Data for one replicate of one treatment were dropped because most of the larvae died of an unidentified cause.

Table 6.1. Schedule of introduction of the 24-hour old *Coleomegilla maculata* larvae.

| treatment | | day | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| population | total number of larvae per vial | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| control | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| mixed-age | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 4 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| | 5 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 7 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 |
| | 13 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 26 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| | 39 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | 52 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| even-aged | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 13 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 39 | 39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

The number of larvae present in each vial was recorded daily, including larvae that were introduced on that day for the mixed-age populations. Survivorship was expressed as the percentage of pupae collected from each vial to the total number of larvae introduced. Because the survivorship curves for both types of population were curvilinear, they were statistically analyzed by non-linear regression using the DUD method (=Doesn't Use Derivatives, also called the false position method) in the NLIN procedure of the Statistical Analysis System (SAS Institute, 1988, pp. 675-712). The following exponential equation was fitted to the data for both types of population:

$$S_P = S_1 \cdot P^c \qquad (6.1)$$

where $S_P$ is the predicted survivorship for P, the number of larvae introduced per vial, $S_1$ is the estimated survivorship of a single larva, and $c$ represents the strength of density-dependent larval cannibalism. If estimates of $c$ are not statistically different from 0, the predicted survivorship across population levels are not different from the survivorship of a singly reared larva. If only one larva survives in each population level, $c$ reaches a maximum value of -1. Estimates of $c$ can be compared between aged-structured and even-aged populations and for statistical differences with boundaries using asymptotic 95 % confidence intervals.

Individual pupal weights were recorded to test for differences within and between populations. A weighed ANCOVA was performed on mean pupal weights calculated for each replicate, using the initial number of larvae as the covariate and the number of pupae collected in each replicate as the weighing variable.

## 6.3 Results

As predicted, cannibalism was present in both mixed-age and even-aged populations of *C. maculata* larvae, although it was significantly higher in the former (Table 6.2, Fig. 6.1). The non-linear model specified here fitted the data well, with 87 % and 62 % of the variation in survivorship explained for mixed-age and even-aged populations, respectively. The survivorship of singly reared larvae was 100 % ± 0.0 (mean ± s.e.) and estimates for this parameter were not significantly different from this experimental value for both types of population (Table 6.2). Estimates of *c*, the parameter describing the strength of density-dependent cannibalism, were significantly different from -1 for both mixed-age and even-aged populations (Table 6.2). The value of -1 is the lower boundary for parameter *c* in the extreme situation where one larva cannibalizes all the others, irrespective of the number of larvae introduced per vial. The lower curve in figure 6.1 shows how survivorship varies with respect to population size for this hypothetical situation and basically represents the behavior of the model, a monotonic decelerating function of the independent variable.

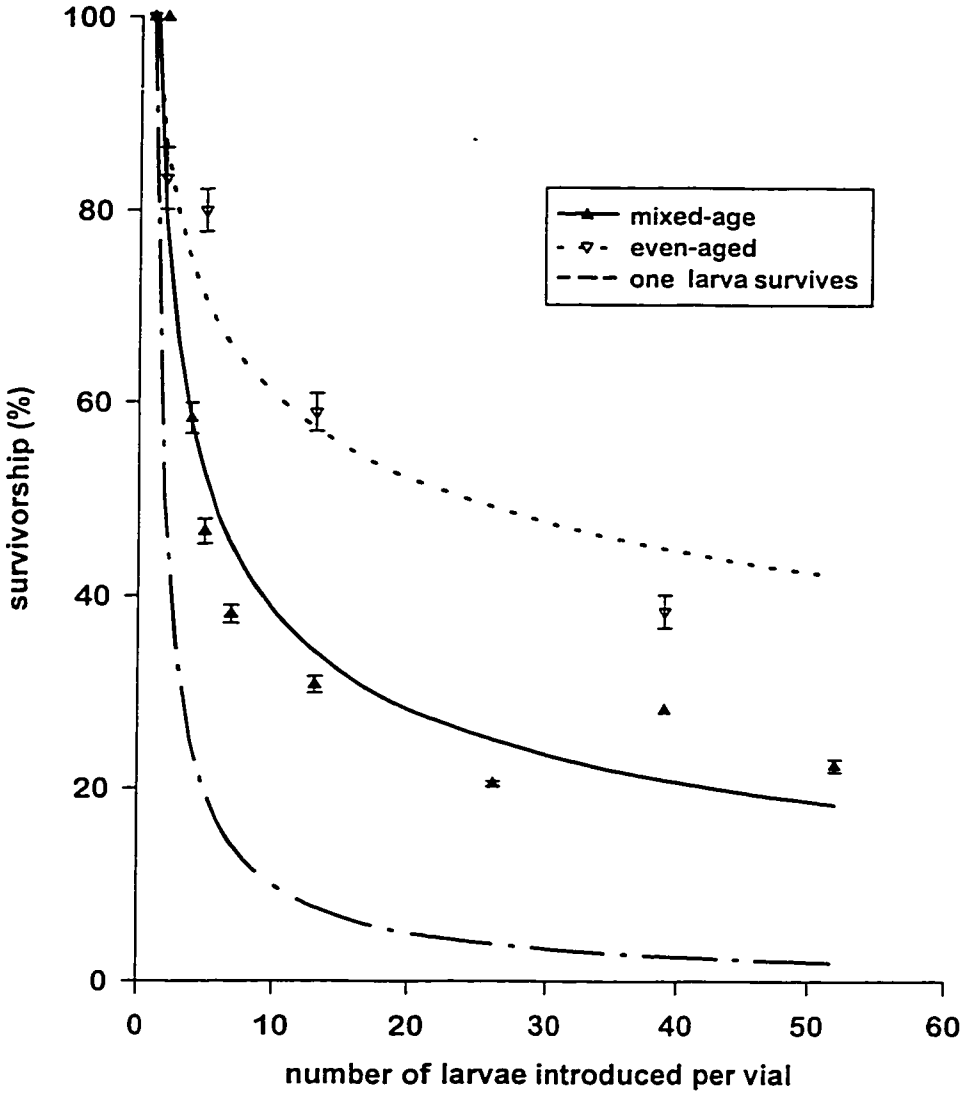
Table 6.2. Non-linear regressions on survivorship data for mixed-age and even-aged populations of *C. maculata* larvae. Data were fitted to the equation: $S_P = S_I \cdot P^c$. $S_P$ : predicted survivorship for P, the total number of larvae introduced per vial, $S_I$ : estimated survivorship of a singly reared larva, *c* : strength of density-dependent cannibalism.

| mixed-age | | | | |
|---|---|---|---|---|
| Source | *d.f.* | *SS* | | $R^2$ |
| Regression | 2 | 86 314.72 | | 0.87 |
| Residuals | 24 | 3 013.10 | | |
| Parameter | Estimate ± 1 s.e. | Asymptotic 95% confidence interval | | |
| | | Lower | Upper | |
| $S_I$ † | 108.67 ± 5.59 | 97.12 | 120.22 | |
| *c* § | -0.45 ± 0.04 | -0.53 | -0.37 | |
| even-aged | | | | |
| Source | *d.f.* | *SS* | | $R^2$ |
| Regression | 2 | 84 478.24 | | 0.62 |
| Residuals | 13 | 3 991.60 | | |
| Parameter | Estimate ± 1 s.e. | Asymptotic 95% confidence interval | | |
| | | Lower | Upper | |
| $S_I$ | 101.36 ± 9.33 | 83.38 | 119.35 | |
| *c* | -0.22 ± 0.05 | -0.34 | -0.11 | |

† the control mean (s.e.) is 100.0 (0.0).

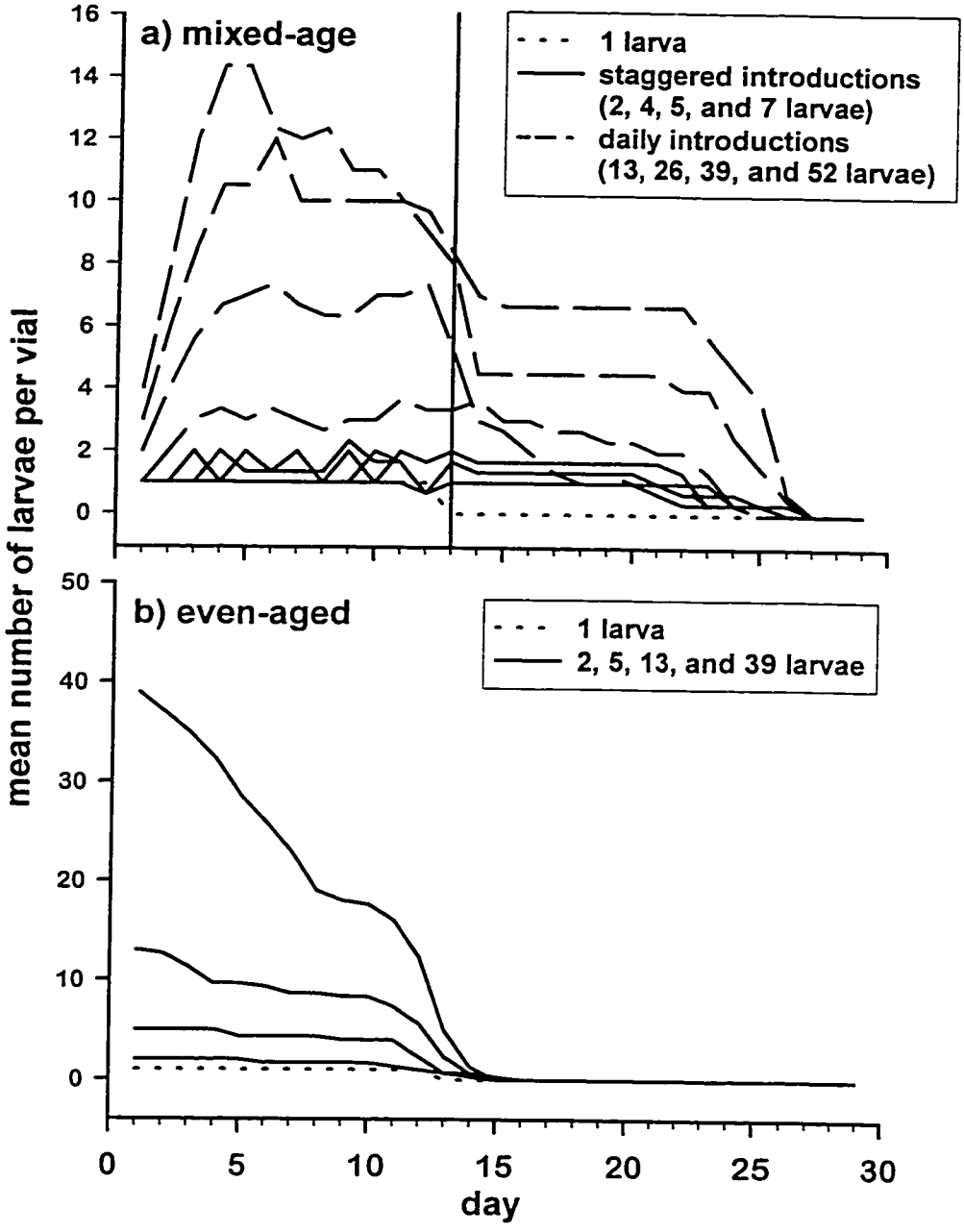
§ *c* is bound between 0 (no mortality due to cannibalism) and -1 (one larvae cannibalized all the others).

Figure 6.1. Survivorship curves for mixed-age and even-aged populations of *C. maculata* larvae (mean ± s.e.). Curves were fitted using non-linear regressions (*see text;* Table 6.2). The bottom curve represents the situation where all but one larva succumb to cannibalism for all population level.


100
80
60
40
20
0
survivorship (%)
mixed-age
even-aged
one larva survives
0
10
20
30
40
50
60
number of larvae introduced per vial

When the change in the number of larvae in each population level is plotted with respect to time (Fig. 6.2a+b), several patterns emerge. First, when the introductions of larvae were staggered to generate mixed-age populations, newly-introduced larvae were discovered and cannibalized within 24 hours (Fig. 6.2a), confirming the preliminary observations that the size of the vials used in this experiment was small enough to minimize the effects of increasing rates of encounters with increasing densities. Second, when mixed-age populations were generated by daily introductions at a constant rate, their temporal dynamics indicate that there was a time-lag of 3-4 days before the onset of cannibalism, i.e., the population size reached in these vials after this time-lag is roughly the product of the number of larvae introduced daily and this time-lag. Once this time-lag was over, virtually no larva could enter the established populations and escape cannibalism until individuals in these populations left the system as pupae (Fig. 6.2a, *right of the vertical line indicating the last day of introductions*). Between the onset of cannibalism in those mixed-age populations and the last day of introductions, the number of larvae remained fairly constant for the lower population levels generated by daily introductions (13 and 26), but started to decline at the higher levels (39 and 52). During this period, these populations assumed a nearly even-aged distribution and behaved in a similar manner to the experimentally generated even-aged populations of comparable sizes (Fig. 6.2b). In these latter populations, the number of larvae decreased with time at an increased rate as initial size increased.

Figure 6.2. Change in the number of individuals with time in (a) mixed-age and (b) even-aged populations of *C. maculata* larvae. Means of three replicates are shown. Error bars are not drawn for clarity. In general, the standard errors within each population level increased as population size increased, but never exceeded 2.3 and 5.3 for the mixed-age and even-aged populations respectively. The vertical line in the upper graph indicates the last day of introduction for the mixed-age populations.

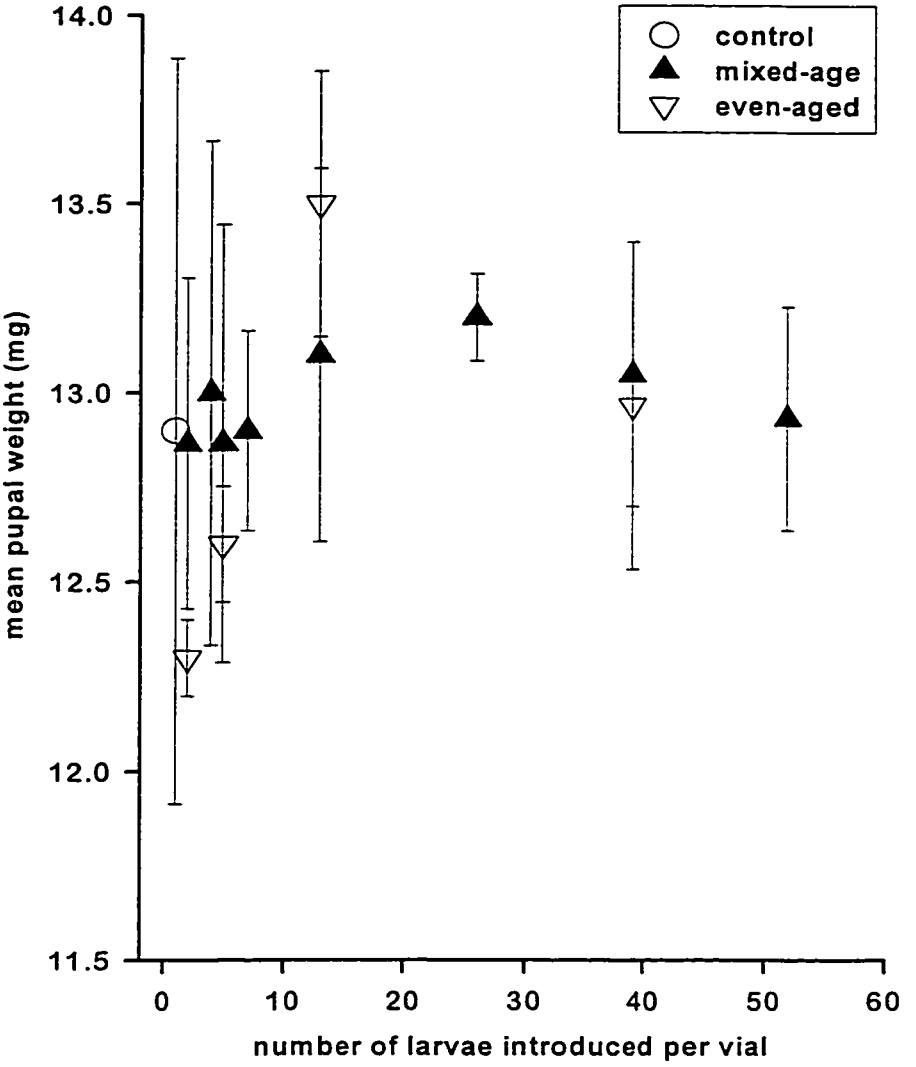

a) mixed-age
1 larva
staggered introductions
(2, 4, 5, and 7 larvae)
daily introductions
(13, 26, 39, and 52 larvae)
b) even-aged
1 larva
2, 5, 13, and 39 larvae
mean number of larvae per vial
day
0
2
4
6
8
10
12
14
16
0
10
20
30
40
50
0
5
10
15
20
25
30

Mean individual size, as measured by pupal weight, did not differ significantly between mixed-age and even-aged populations, and was not a significant linear function of population size (Table 6.3). Though there was a trend in both types of population for mean pupal weights to initially increase with population size and then decrease (Fig. 6.3). However, quadratic terms were not significant when polynomial regressions were performed on these data. Moreover, the overall mean of pupal weights in the experimental populations (12.94 mg ± 0.11) was similar to the mean pupal weight of the singly reared larvae (12.90 mg ± 0.99). Hence, it appears that each individual contributed equally to the final population biomass, irrespective of population size.

Table 6.3. ANCOVA on mean pupal weights with the number of larvae introduced as the covariate. Data were weighed using the number of pupae collected from each vial.

| Source | *d.f.* | *MS* | *F-value* | *P* |
|---|---|---|---|---|
| Number of larvae introduced | 1 | 0.267 | 0.13 | 0.7179 |
| Populations | 1 | 0.433 | 0.21 | 0.6463 |
| Interaction | 1 | 0.032 | 0.02 | 0.9003 |
| Error | 31 | 2.014 | | |

Figure 6.3. Mean weights (± SE) of pupae from mixed-age and even-aged populations of *C. maculata*.


14.0
13.5
13.0
12.5
12.0
11.5
mean pupal weight (mg)
0
10
20
30
40
50
60
number of larvae introduced per vial
control
mixed-age
even-aged

## 6.4 Discussion

In general, experiments on cannibalism focus on density-dependent mechanisms, such as rate of encounters, or on alternative food levels (*references* in Polis, 1981) as factors influencing the degree of interference mortality among individuals in a population. Here, although I investigated how age, and thus, relative size distribution affect cannibalism rates, as other published studies have done before (*references in* Polis [1981] and Fox [1975]), I minimized encounter rate and food shortage effects, which can be confounding factors. In doing so, it was possible to fit to the survivorship data a simple exponential model which behaves as a monotonic decelerating function of population size. Although the model does not provide insights on why the strength of cannibalism is higher in mixed-age as compared to even-aged populations (Table 6.2), or less than in the situation presented in figure 6.1 where only one cannibal survives, it indicates that once encounter rates and food shortage are controlled for, survivorship decreases at a decreasing rate as population size increases, whatever the underlying mechanism. Hence, it is a basic model on which more complex ones may be based. Other models of density-dependent mortality (e.g., Bellows, 1981) failed to fit the situation where only one cannibal survives, a likely situation when alternate food level is low.

Although cannibalism was less severe in the even-aged populations compared to the mixed-age populations, confirming the hypothesis that density-dependent mortality is stronger when there is an age and size differential in the individuals of a population, survivorship decreased to a predicted 44.9 % at the highest population level tested, 39 larvae per vial (Fig. 6.1). This means that roughly half the larvae converted the other half into their own body mass, even if they were of the same age and in the presence of a super-abundant alternative food supply. Why this did not occur at smaller population sizes despite the fact that even small larvae could search the whole area of the vial may have to do with cannibalism events occurring when larvae are molting, and thus incapable of defending themselves (Hodek, 1970). Since larvae are likely to have slightly different individual growth rates, and thus molting schedules, the probability of encountering a molting larva may increase as population size increases. The same situation is likely to occur in mixed-age populations when larvae have reached a certain age or size. After a time-lag of about 3-4 days after the initiation of the experiment, not only the larvae already present in the vials prevented the newly-introduced larvae from developing, they also cannibalized each other at the higher rates of introduction (Fig. 6.2a).

The results of the present study taken together suggest that larvae of *C. maculata* will prey on conspecifics whenever the opportunity arises, even when food is not limiting. A similar conclusion was reached in other studies involving

other species of insects (Dial and Alder, 1990; Reed *et al.*, 1996) or organisms (Van den Bosch and Santer, 1993). In contrast, Agarwala and Dixon (1992) showed in laboratory trials that larval cannibalism in the ladybird *Adalia bipunctata* (L.) related inversely to the abundance of aphids. However, their experiment lasted 24 hours and hence did not take into account interactions among larvae during the whole duration of their development. The possibility exists that in the present experiment, *C. maculata* larvae preferred conspecifics over the artificial diet they were offered, although none of the control larvae died and their weight at pupation did not differ significantly from individuals that had eaten conspecifics.

Diet preference is obviously another factor to consider as it affects cannibalism rates (*references in* Polis, 1981). However, had I experimented with aphids as an alternative food source, the problem would not have been necessarily resolved since different aphid species or aphids of the same species feeding on different host plants are not equally suitable for growth (Blackman, 1967; Hodek, 1967, 1973). Hence, the strength of cannibalism may change depending on the alternative food offered, but should not be confounded with levels of alternative food. In order to compare the effects of each factor, one would also have to control for encounter rates with both conspecifics and the alternative food source.

In the confines of the small vials in which the present experiment was performed, laying eggs synchronously appears to be more profitable than laying

eggs at a constant rate, lending further support to the optimal strategy proposed by Hemptinne *et al.* (1992) in which ladybird females concentrate oviposition early in the existence of an aphid colony. In the field however, where the probability of encounters varies with density, it is likely that it would be more profitable for ladybird females to choose the second strategy, if they are not constrained by time, i.e., larval duration is short relative to the duration of aphid colonies. Otherwise, females would have to adjust the size of their egg clusters (Kindlmann and Dixon, 1993) or leave an exploited aphid colony so as to minimize cannibalism. Only the appropriate field experiment would help resolve this issue further. Using key-factor analysis, Osawa (1993) showed that larval mortality in the ladybird *Harmonia axyridis* was density-dependent in the field. It is possible that ladybird beetles do not behave optimally and rely on cannibalism as a "life-boat" strategy *sensu* Giese (1973; *cited in* Polis [1981]).

# — Chapter VII —

## Optimal Foraging in Ladybird Beetles: Test of a Model

### 7.1 Introduction

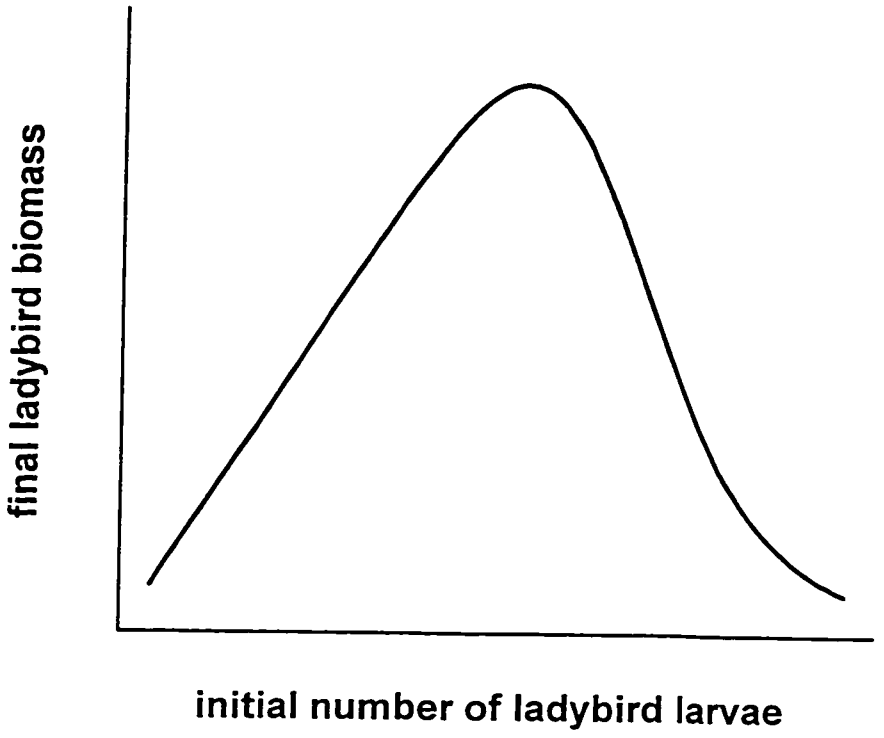
Optimal foraging theory is a tool to answer the question of what animals should do to gather food in the most efficient way possible so as to maximize fitness (Crawley and Krebs, 1992). When considering ladybird beetles feeding on aphids, the question may be more precisely stated as: "how many eggs a female should lay in the vicinity of an aphid colony to maximize the number of offspring that will survive at the end of the existence of this aphid colony?" This optimization problem arises for two main reasons: 1) given the high reproductive rate of aphids, each aphid removed by predation early in the existence of a colony represents in fact several hundreds of individuals which will not be present a few days later, and 2) as the ladybird larvae mature, their requirement for food increases exponentially. Hence, laying too many eggs in an aphid colony is immediately conducive to strong competition among the larvae, which is relaxed by cannibalism.

This optimization problem has been recently framed in a series of equations modeling the temporal dynamics of both the aphids and the ladybird larvae present in the same colony (Kindlmann and Dixon, 1993). One of the main outcomes of this

model is that there is effectively an optimal initial number of larvae which will maximize the final biomass of ladybirds at the end of the existence of an aphid colony (Fig. 7.1). The model also indicates that this optimum number is dependent on the attack rate upon the aphids and on the preference of ladybird larvae for aphids as opposed to conspecifics. What the model did not explore, however, is the effect of increasing the size of the aphid colony on the location of the optimum. In the present study, I examined in the laboratory aphid colony size effect using caged sorghum plants inoculated with increasing initial numbers of the aphid *Rhopalosiphum maidis*. In these cages, a range of initial number of larvae of the ladybird *Coleomegilla maculata* have been introduced to determine which initial conditions would produce the greater number of individuals alive after a fixed period of time, i.e. two weeks, which is about the duration of the larval development for this species. It was hypothesized that the optimum initial number of ladybird larvae would increase with an increase in the size of the aphid colony.

The model of Kindlmann and Dixon (1993) makes yet the interesting prediction that the reduction in the number of aphids when the optimum is reached is relatively small. This, also, has been tested. If true, though, it may be predicted that what would be considered as to be the optimal strategy for the ladybirds would not be in favor of the plant, i.e. the effects of predation by the optimal number of ladybird larvae will not result in a trophic cascade.

Figure 7.1 The final biomass of ladybirds at the end of the existence of an aphid colony in relation to the initial number of ladybird larvae as predicted by the model of Kindlmann and Dixon (1993).


final ladybird biomass
initial number of ladybird larvae

## 7.2 Material and methods

### 7.2.1 Experimental

The experiment was conducted in an environmental chamber (25 °C, 70 % r.h., and 16L:8D). Sorghum plants (*Sorghum vulgare*; Cargill hybrid, common soudangrass) were grown singly in 1 L polystyrene pots with no bottom, filled with vermiculite pre-soaked in full-strength Hoagland's solution. The pots were placed in 500 ml plastic cups so the plants could be fed weekly by filling the space between the cup and the pot with full-strength Hoagland's solution (250 ml/week). The top of the pots were fitted with a circular piece of polystyrene to anchor a wire frame which supported the cage itself. This piece had a hole in its center through which the plant could grow. The cages, made of fine-mesh polyester, were of the tube type (75 cm in height), open at one end to slide over the wire frame and close at the top with a circular piece of clear polyvinyl (20 cm in diameter). The cages were placed over the plants only after they were inoculated with aphids, that is 2 weeks after sowing. Large rubber bands were used to secure the base of the cages on the pots.

Five levels of inoculation were performed ($N_0$ = 0, 1, 2, 5, and 10) with immature aphids (L3-L4) from a clonal culture. The plants selected for the

experiment were all at the same growth stage, i.e. with the blade of the 5th leaf protruding out of the whorl. Plants were sampled at regular intervals after inoculation (every week for 3 weeks) to measure the increase in total dry biomass and to count the number of aphids present (in the absence of ladybirds).

One week after the plants were inoculated with aphids, 24-hour old ladybird larvae from females reared in the laboratory were placed in the cages at five densities ($K$ = 1, 2, 4, 8, and 16) following a design fully-crossed with all levels of aphid inoculation (except for $N_0 = 0$). The experiment was ended 2 weeks after the ladybird larvae were introduced. The remaining ladybirds were then counted and sorted by their development stage. The remaining aphids were counted and sorted into apterae and winged individuals. The plants, clean of aphids, were oven-dried and weighed.

Each treatment was repeated 3 times in the same environmental chamber. Because of the large number of treatments involved in this experiment, replicates were performed at different dates and thus, blocking was over time.

### 7.2.2 Statistical analyses

The final ladybird biomass predicted by the optimal foraging model (Fig. 7.1) can be approximated by an expression with a quadratic term since one of its essential features is that there is an optimum initial number of ladybird larvae which maximizes the final biomass of the predators. A response surface analysis can thus be used to fit a quadratic response surface to the data collected here where the initial number of aphids was varied as well as the initial number of ladybird larvae. This methodology is appropriate to find a maximum in the response variable and, if there is one, to determine if the optimum resides within the range of the factors under study (Petersen, 1985). Briefly, the first step is to perform a one-way ANOVA to test whether there is a significant treatment effect (by definition, each combination of the levels of each factor is a treatment). If so, the sum of squares associated with the treatment effect is then partitioned into the sum of squares calculated from fitting a quadratic regression to the data and the sum of squares unexplained by the regression, referred to as the lack of fit. The general expression for a quadratic regression for two factors is:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1^2 + \beta_4 x_1 x_2 + \beta_5 x_2^2 + \varepsilon \qquad (7.1)$$

where y is the response variable, βs are the regression parameters to be estimated, $x_1$ and $x_2$ are the regressor, or independent, variables, and ε is the experimental error term. There is 5 degrees of freedom associated with the regression. This number is subtracted from the total number of degrees of freedom for the treatment effect to give the number of degrees of freedom associated with the lack of fit. The RSREG procedure of SAS (SAS Institute, 1988, pp. 877-896) tests both the significance of the regression and the lack of fit. The procedure also performs a canonical analysis which estimates an optimum response ridge, i.e., the combination of the levels of the factors which maximize the response. To center the design of the experiment with respect to the ranges of the initial number of ladybird larvae ($K$ = 1, 2, 4, 8, and 16) and of the initial number of aphids ($N_0$ = 1, 2, 5, and 10), both $K$ and $N_0$ were transformed to their natural logarithm prior to analysis.

Response surface analysis was also applied to the decrease in peak aphid number owed to the ladybirds as expressed by the ratio of the $\log_{10}$ number of aphids in the presence of the ladybirds at the end of the experiment to the $\log_{10}$ number of aphids in their absence (Kindlmann and Dixon, 1993). This relative measure of the impact of the ladybirds on the aphids ensure a meaningful comparison among the treatments since it measures the capacity of the ladybirds to counteract the intrinsic exponential nature of aphid population growth. Ratios were computed by blocks.

## 7.3 Results

### 7.3.1 Ladybirds

Table 7.1 gives the final numbers of ladybirds resulting from the different combinations of initial number of larvae and aphids. The results are in agreement with the prediction of the model of Kindlmann and Dixon (1993) that there is an optimum initial number of ladybird larvae which maximizes the final number of individuals, as shown by the fitted quadratic response surface in figure 7.2. The canonical analysis indicated that increasing the initial number of aphids did not substantially increase the initial number of ladybird larvae which maximizes the final number (Fig. 7.3a), although it did increase the final number of ladybirds when the optimum number of larvae was reached (Fig. 7.3b). Within the design of this experiment, the optimum number of ladybird larvae was rapidly reached and occurred between 4 and 5 individuals (Fig. 7.3a).

If the optimum number of ladybird larvae was relatively insensitive to the number of aphids initiating a colony, increasing the initial number of aphids resulted in significantly higher survivorship in treatments at, or below, the optimum ($K \leq 4$: $d.f. = 3$, $G = 10.375$, $p = 0.018$), but not in treatments above it ($K > 4$: $d.f. = 3$, $G = 3.063$, $p = 0.382$) (Fig. 7.4). The examination of individual treatment means (Table 7.3) shows that increasing the initial number of aphids resulted in survivorship reaching, or approaching, 1.0 for cohorts of ladybird larvae below, or at, the optimum, whereas survivorship dropped drastically for cohorts above the optimum.

Table 7.1 Mean number of ladybirds per cage at the end of the experiment.

| | | Initial number of ladybird larvae | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 4 | 8 | 16 |
| initial number of aphids | 1 | 1.0 (0.0)† | 1.3 (0.3) | 1.3 (0.3) | 1.3 (0.3) | 1.0 (0.0) |
| | 2 | 1.0 (0.0) | 1.7 (0.3) | 2.0 (0.6) | 1.3 (0.3) | 1.0 (0.0) |
| | 5 | 1.0 (0.0) | 2.0 (0.0) | 3.0 (0.6) | 2.7 (0.7) | 1.3 (0.3) |
| | 10 | 1.0 (0.0) | 1.7 (0.3) | 3.7 (0.3) | 2.0 (0.6) | 2.0 (0.6) |

† standard error

Figure 7.2. Response surface for the number of ladybirds at the end of the experiment as predicted from the initial number of ladybird larvae ($K$) and the initial number of aphids ($N_0$). The fitted quadratic regression is: final number = $0.701 + 0.327\ln(N_0) + 1.514\ln(K) - 0.060(\ln(N_0))^2 + 0.162(\ln(N_0)\ln(K)) - 0.570(\ln(K))^2$ ($R^2 = 0.47$, $p < 0.0001$). ANOVA results are given in Table 7.2. The predicted maximum number of ladybirds at the end of the experiment is 3.2 and outside the design at the coordinate ($K = 8.4$: $N_0 = 277.2$).

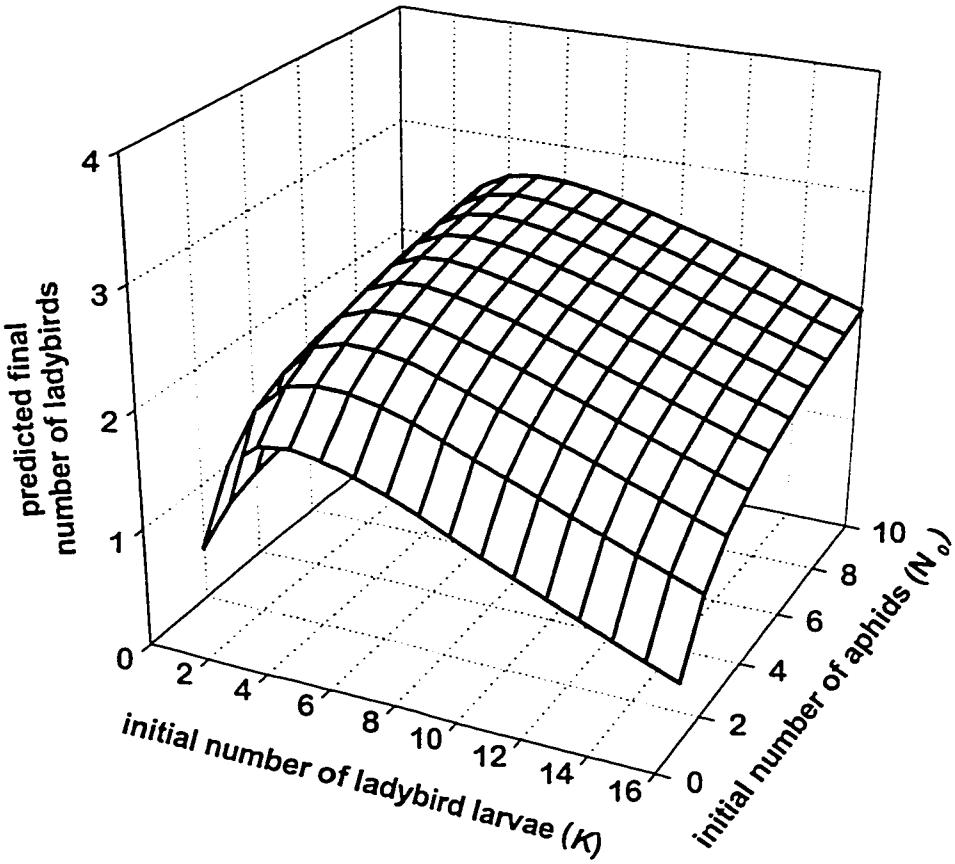
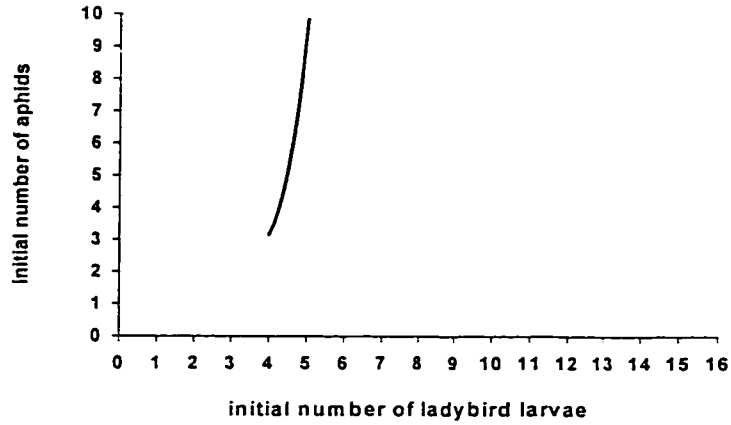

predicted final number of ladybirds
0
1
2
3
4
initial number of ladybird larvae (K)
2
4
6
8
10
12
14
16
initial number of aphids ($N_o$)
0
2
4
6
8
10

Table 7.2 ANOVA on final number of ladybirds. The initial number of ladybird larvae and the initial number of aphids were ln-transformed before fitting the quadratic surface.

| Source | *d.f.* | *SS* | *MS* | *F* | *P* |
|---|---|---|---|---|---|
| Model | 21 | 31.767 | 1.513 | 3.69 | 0.0002 |
| Block | 2 | 0.433 | 0.217 | 0.53 | 0.5935 |
| Treatment | | | | | |
| fitted surface | 5 | 22.570 | 4.514 | 11.02 | <0.0001 |
| lack of fit | 14 | 8.763 | 0.626 | 1.53 | 0.1479 |
| Error | 38 | 15.567 | 0.409 | | |

Figure 7.3. The estimated ridge of the response surface as viewed a) from above and b) from the side.

a)


initial number of aphids
0
1
2
3
4
5
6
7
8
9
10
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
initial number of ladybird larvae

b)

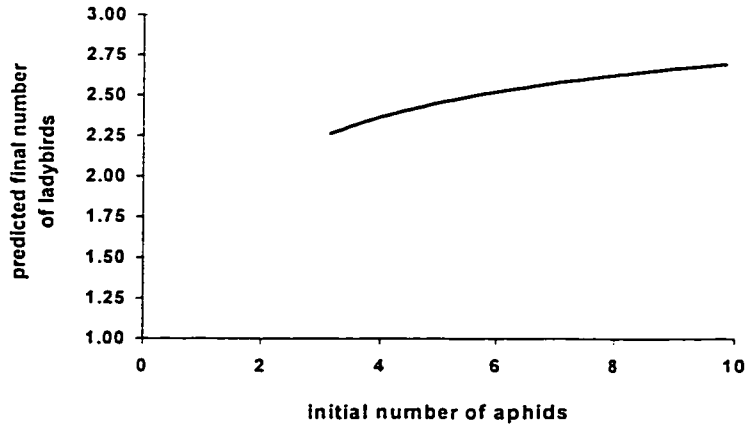

predicted final number of ladybirds
3.00
2.75
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0
2
4
6
8
10
initial number of aphids

Figure 7.4 Proportion of ladybirds surviving at the end of the experiment in the treatments at, or below, the optimum initial number of larvae ($K \leq 4$) and above the optimum ($K > 4$). Different letters above the bars indicate significant differences between levels of initial number of aphids using multiple $G$ tests ($K \leq 4$: $d.f. = 3$, $G = 10.375$, $p = 0.018$; $K > 4$: $d.f. = 3$, $G = 3.063$, $p = 0.382$). Survivorship at, or below, the optimum significantly different from above ($d.f. = 1$, $\chi^2 = 121.565$, $p < 0.0001$).

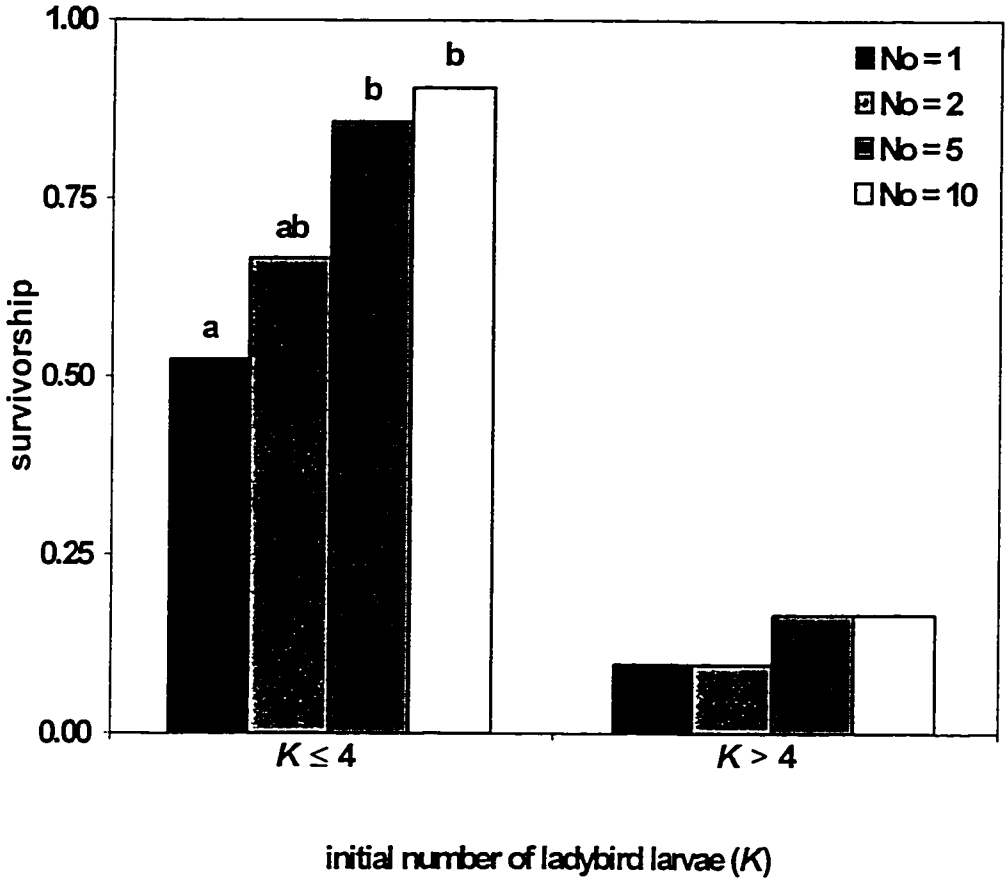

1.00
0.75
0.50
0.25
0.00
survivorship
a
ab
b
b
No = 1
No = 2
No = 5
No = 10
K ≤ 4
K > 4
initial number of ladybird larvae (K)

Table 7.3 Survivorship of ladybirds at the end of the experiment. Means and their standard errors (in brackets) are shown.

| | | initial number of ladybird larvae | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 4 | 8 | 16 |
| initial number of aphids | 1 | 1.00 (0.00) | 0.67 (0.17) | 0.33 (0.08) | 0.17 (0.04) | 0.06 (0.00) |
| | 2 | 1.00 (0.00) | 0.83 (0.17) | 0.50 (0.14) | 0.17 (0.04) | 0.06 (0.00) |
| | 5 | 1.00 (0.00) | 1.00 (0.00) | 0.75 (0.14) | 0.33 (0.08) | 0.08 (0.02) |
| | 10 | 1.00 (0.00) | 0.83 (0.17) | 0.92 (0.08) | 0.25 (0.07) | 0.13 (0.04) |

Proportionally more ladybirds reached adulthood at the end of the experiment in cohorts below, or at, the optimum ($K \leq 4$) compared to cohorts above the optimum ($K > 4$) (Fig. 7.5; $d.f. = 1$, $\chi^2 = 18.980$, $p < 0.0001$). Increasing the initial number of aphids did not affect the proportion of ladybirds reaching adulthood within cohorts at, or below, the optimum ($K \leq 4$: $d.f. = 3$, $G = 2.375$, $p = 0.498$) or within cohorts above the optimum ($K > 4$: $d.f. = 3$, $G = 3.045$, $p = 0.385$).

### 7.3.2 Aphids

In the absence of ladybirds, the growth of the aphid colonies on the sorghum plants was tremendous, reaching in 3 weeks a mean of 1 624 (s.e. = 171) individuals when initiated with one aphid and a mean of 7 027 (s.e. = 806) individuals when initiated with 10 aphids. The growth of the aphid colonies was not exponential during all of these 3 weeks. There was a lag-phase during the first week after inoculation, followed by exponential growth during the second week, and, finally, a decrease in the growth rate of the colonies was evident during the last week of the experiment, as shown in figure 7.6 by the significant cubic regressions fitted to the $\log_{10}$-transformed data. The initial lag-phase in the growth of the aphid colonies is the consequence of the colonies being initiated by immature aphids (L3-L4). The decrease in the growth rate of the aphid colonies during the last week is indicative that the peak numbers of individuals were somehow reached earlier than on the last day of the experiment.

Figure 7.5. Proportions of adult ladybirds at the end of the experiment in the treatments at, or below, the optimum initial number of larvae ($K \leq 4$) and above the optimum ($K > 4$). No significant differences found within groups using $G$ tests ($K \leq 4$: $d.f. = 3$, $G = 2.375$, $p = 0.498$; $K > 4$: $d.f. = 3$, $G = 3.045$, $p = 0.385$). Proportion of adults at, or below, the optimum significantly different from above ($d.f. = 1$, $\chi^2 = 18.980$, $p < 0.0001$).

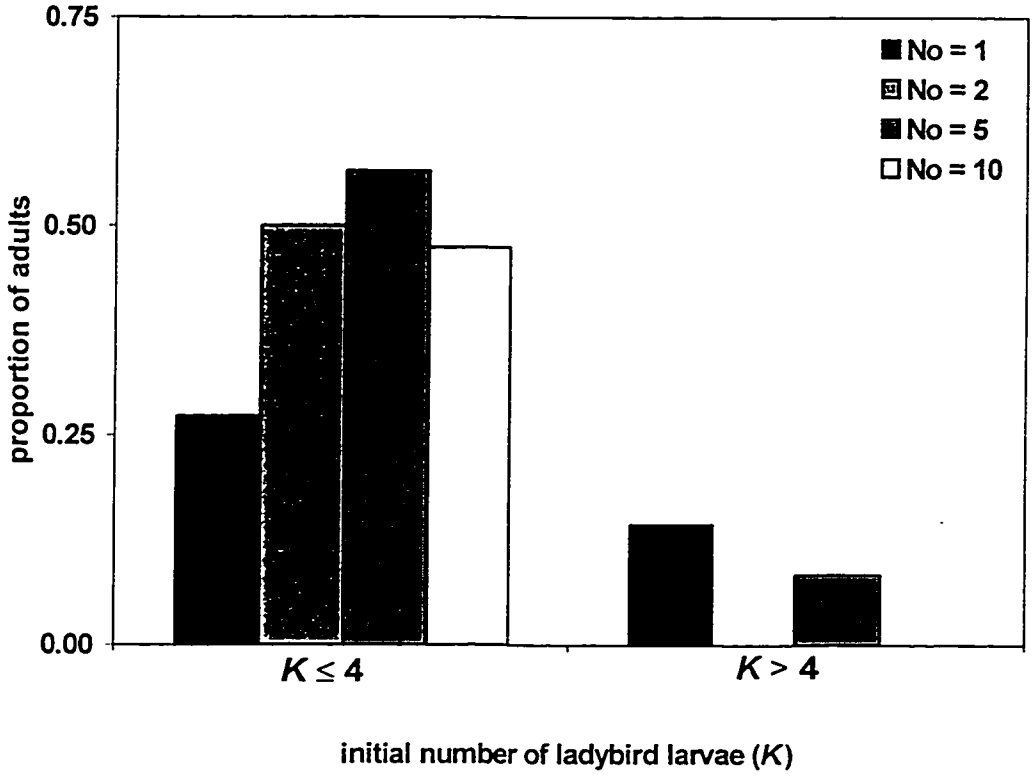
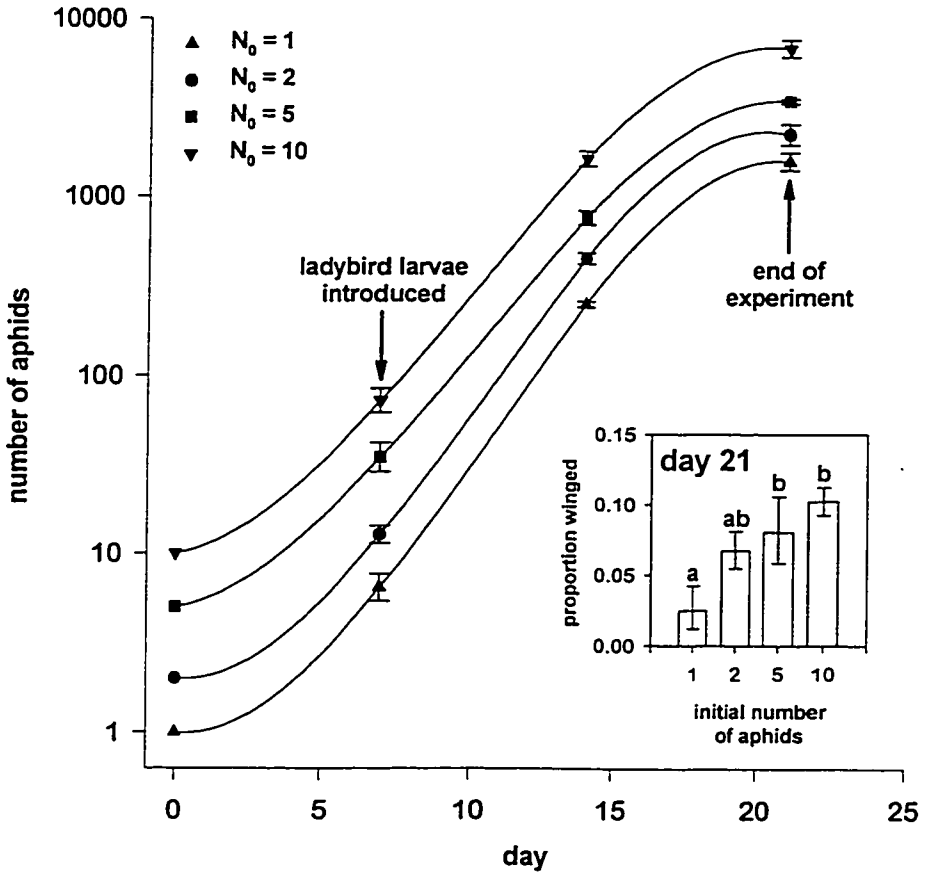

0.75
0.50
0.25
0.00
proportion of adults
No = 1
No = 2
No = 5
No = 10
K ≤ 4
K > 4
initial number of ladybird larvae (K)

Figure 7.6 Growth of aphid colonies initiated with different numbers of individuals ($N_0$) in the absence of ladybirds (time of introduction of ladybird larvae on the other experimental plants indicated). Means and standard errors are shown along with cubic regression lines performed on $\log_{10}$-transformed data (all regressions significant, $p < 0.0001$). Note the logarithmic scale on the $y$-axis. Winged individuals were observed only on the last day of the experiment (day 21). Their proportions to the total number of individuals are shown in the inserted bar graph. Different letters above the bars indicate significant differences ($p = 0.05$) between initial inoculation levels as established by a Waller-Duncan test performed on arcsin square-root transformed data (back-transformed means and standard errors are shown).


10000
1000
100
10
1
number of aphids
$N_0 = 1$
$N_0 = 2$
$N_0 = 5$
$N_0 = 10$
ladybird larvae introduced
end of experiment
0
5
10
15
20
25
day
day 21
proportion winged
0.15
0.10
0.05
0.00
a
ab
b
b
1
2
5
10
initial number of aphids

The presence of winged individuals on the last count, but not on previous counts, is a confirmation of the peak number being reached before the end of the experiment. However, the small proportions of winged individuals at the end of the experiment, never more on average than 10.2 %, suggest that peak numbers were reached near the last day. It is likely that peak numbers were reached closer to the last day as fewer aphids initiated the colonies since the proportion of winged individuals also decreased in these colonies (Fig. 7.6, *inserted bar graph*).

The impact of the ladybird larvae on the growth of the aphids colonies was computed as the ratio of the $\log_{10}$ number of aphids in the presence of the ladybirds to the $\log_{10}$ number of aphids counted at the end of the experiment in the absence of the ladybirds, including the winged individuals. Since these winged individuals could not escape from the cages, their inclusion is likely to make the final counts closer to the peak numbers.

However, winged individuals were also present in different proportions in cages with ladybirds, an indication that crowding was not the only factor responsible for the decrease in the growth of the aphid colonies towards the end of the experiment (*data not shown*). A decrease in the nutritional quality of the plants, or increased resistance with the maturation of the plant, may also have been important factors. The sorghum variety used in this experiment was sowed in the field several years and the same pattern was observed each time: seedlings were

always moderately to heavily infested by the corn leaf aphid, whereas older plants never harbored colonies, even on the head still enclosed in the whorl. Thus, aphid colonies exposed to the ladybirds in the experimental cages may also have experienced a reduction in their growth rate towards the end of the experiment, despite the release from crowding effected by the presence of ladybirds. The extent of this reduction compared to colonies not exposed to the ladybirds is unknown. In any case, the worst scenario would be that the ratio expressing the decrease in aphid number owed to the ladybirds, as computed here, slightly underestimates the true ratio when more aphids initiated a colony than when a colony is initiated with fewer individuals.

Table 7.4 gives the computed ratios for all the combinations of initial number of aphids and ladybird larvae. A highly significant quadratic regression was fitted to the data (Table 7.5) and figure 7.7 shows the response surface for the ratio. The shape of the surface indicates that for any given initial number of ladybird larvae, the reduction in final aphid number was virtually the same in colonies initiated with different number of aphids. In particular, the estimated ratio ranged between 0.67 and 0.71 when the optimum number of ladybird larvae has been reached, a result similar to the values predicted by the model of Kindlmann and Dixon (1993).

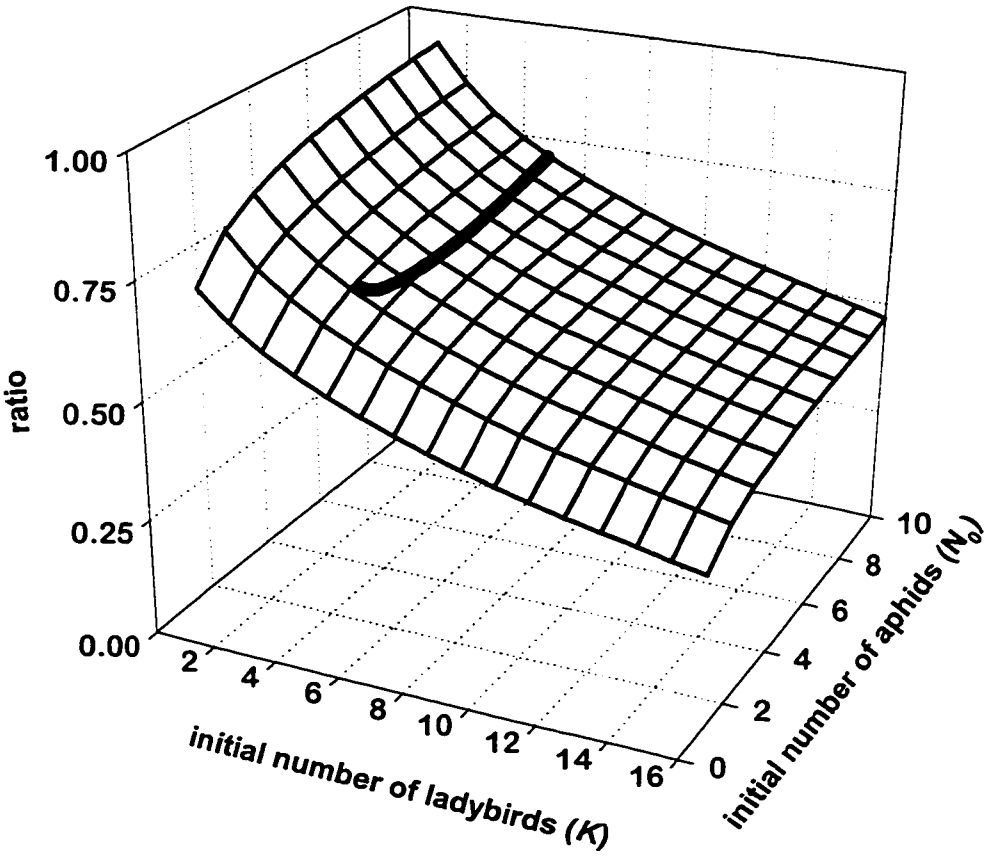
Table 7.4 Ratios of the $\log_{10}$ number of aphids in the presence of ladybirds to the $\log_{10}$ number of aphids in their absence at the end of the experiment. Means and their standard errors (in brackets) are shown.

| | | Initial number of ladybird larvae | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 4 | 8 | 16 |
| initial number of aphids | 1 | 0.67 (0.06) | 0.65 (0.06) | 0.61 (0.06) | 0.41 (0.01) | 0.35 (0.02) |
| | 2 | 0.78 (0.06) | 0.76 (0.02) | 0.68 (0.02) | 0.47 (0.03) | 0.38 (0.04) |
| | 5 | 0.88 (0.02) | 0.81 (0.01) | 0.67 (0.03) | 0.60 (0.02) | 0.41 (0.08) |
| | 10 | 0.95 (0.01) | 0.83 (0.02) | 0.72 (0.01) | 0.61 (0.03) | 0.51 (0.04) |

Table 7.5 ANOVA on ratios of the $\log_{10}$ number of aphids in the presence of ladybirds to the $\log_{10}$ number of aphids in their absence at the end of the experiment. The initial number of ladybird larvae and the initial number of aphids were ln-transformed before fitting the quadratic surface.

| Source | *d.f.* | *SS* | *MS* | *F* | *P* |
|---|---|---|---|---|---|
| Model | 21 | 1.743 | 0.083 | 27.48 | <0.0001 |
| Block | 2 | 0.056 | 0.028 | 9.31 | 0.0005 |
| Treatment | | | | | |
| fitted surface | 5 | 1.638 | 0.328 | 102.39 | <0.0001 |
| lack of fit | 14 | 0.049 | 0.004 | 1.08 | 0.4042 |
| Error | 38 | 0.115 | 0.003 | | |

Figure 7.7 Predicted decrease in peak aphid number as expressed by the ratio of the $\log_{10}$ number of aphids in the presence of ladybirds to the $\log_{10}$ number of aphids in their absence at the end of the experiment. The fitted quadratic regression is: ratio = $0.708 + 0.122\ln(N_0) - 0.070\ln(K) - 0.011(\ln(N_0))^2 - 0.013(\ln(N_0)\ln(K)) - 0.024(\ln(K))^2$ ($R^2 = 0.88$, $p < 0.0001$). The bold line across the surface denotes the response when the optimum initial number of ladybird larvae has been reached (*see* Fig. 7.3a). The range of the response at the optimum is 0.67-0.71.


1.00
0.75
0.50
0.25
0.00
ratio
2
4
6
8
10
12
14
16
initial number of ladybirds (K)
0
2
4
6
8
10
initial number of aphids (N0)

### 7.3.3 Plants

In the absence of both the aphids and the ladybirds, the sorghum plants grew exponentially during the whole duration of the experiment (Fig. 7.8; note the log scale on the *y*-axis). In the presence of aphids, however, a more complex pattern emerged. One week following inoculation, plants with colonies initiated with 2 individuals weighed significantly more than plants free of aphids (Waller-Duncan test at $p = 0.05$). Two weeks following inoculation, plants with colonies initiated with one aphid weighed the most, but significantly more only when compared with plants with colonies initiated with 10 aphids (Waller-Duncan test at $p = 0.05$). On the last day of the experiment, 3 weeks after inoculation, the ranking of the weight of the plants was inversely related to the number of aphids initiating the colonies ($N_0 = 0$ to 10), with the means being transitively different (Waller-Duncan test at $p = 0.05$).

When the ladybirds were present in the cages, their impact on the development of the aphid colonies mediated the final size of the sorghum plants (Fig. 7.9). The overall pattern was that for each level of initial number of aphids, there was a single level, or a threshold level of initial number of ladybird larvae, which resulted in final plant size to reach the size of the plants grown in the absence of both the aphids and the ladybirds and to be larger than the plants with aphid colonies free of ladybirds (*see statistical results in* Fig. 7.9). The larger the initial number of aphids initiating a colony, the larger the initial number of ladybird larvae needed to produce such an outcome.

Figure 7.8 Growth of sorghum plants inoculated with different initial number of aphids ($N_0$) in the absence of ladybird larvae. Ranking and separation of means at each date was achieved using a Waller-Duncan test on $\log_{10}$-transformed data. Different letters indicate significant differences between levels of inoculation at $p$ = 0.05. ANOVA results once block effect removed are as follow: Day 7: *d.f.* = 4, 8, *MS* = 0.0203, $F$ = 4.70, $p$ = 0.0302; Day 14: *d.f.* = 4, 8, *MS* = 0.0792, $F$ = 7.41, $p$ = 0.0085; Day 21: *d.f.* = 4, 8, *MS* = 0.0334, $F$ = 13.67, $p$ = 0.0012.

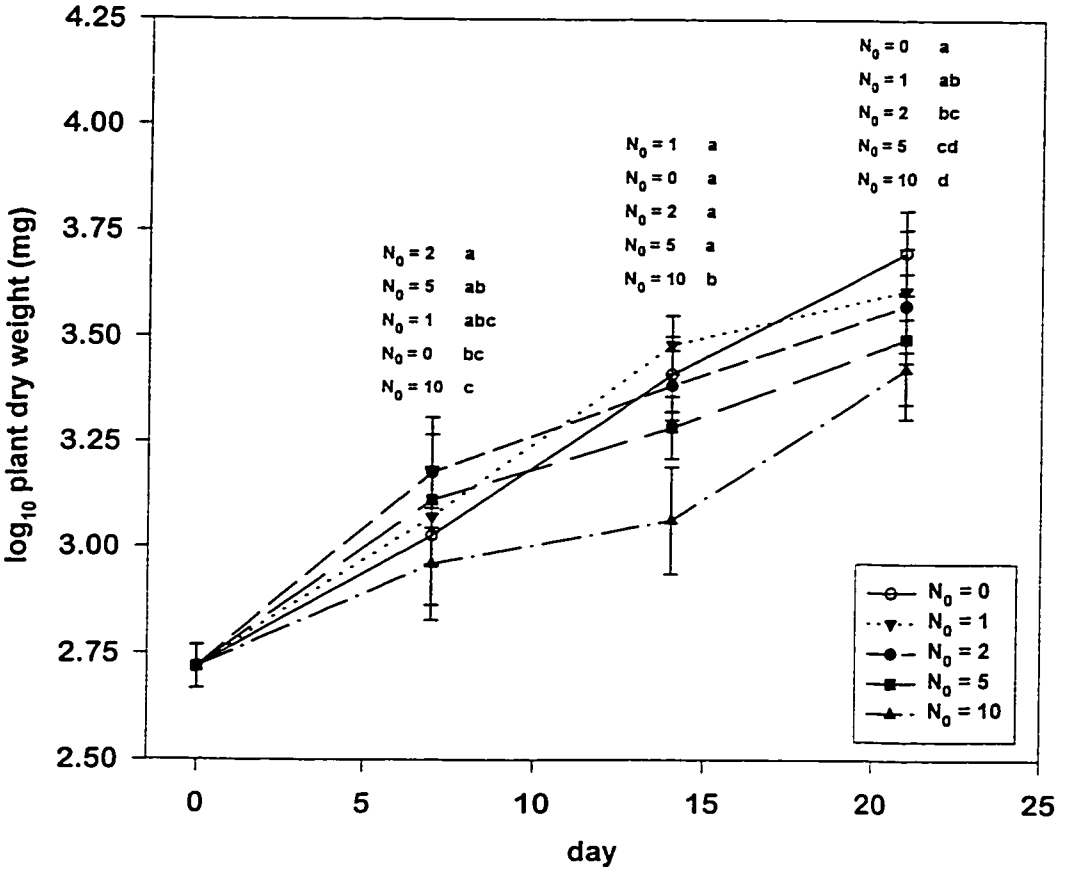
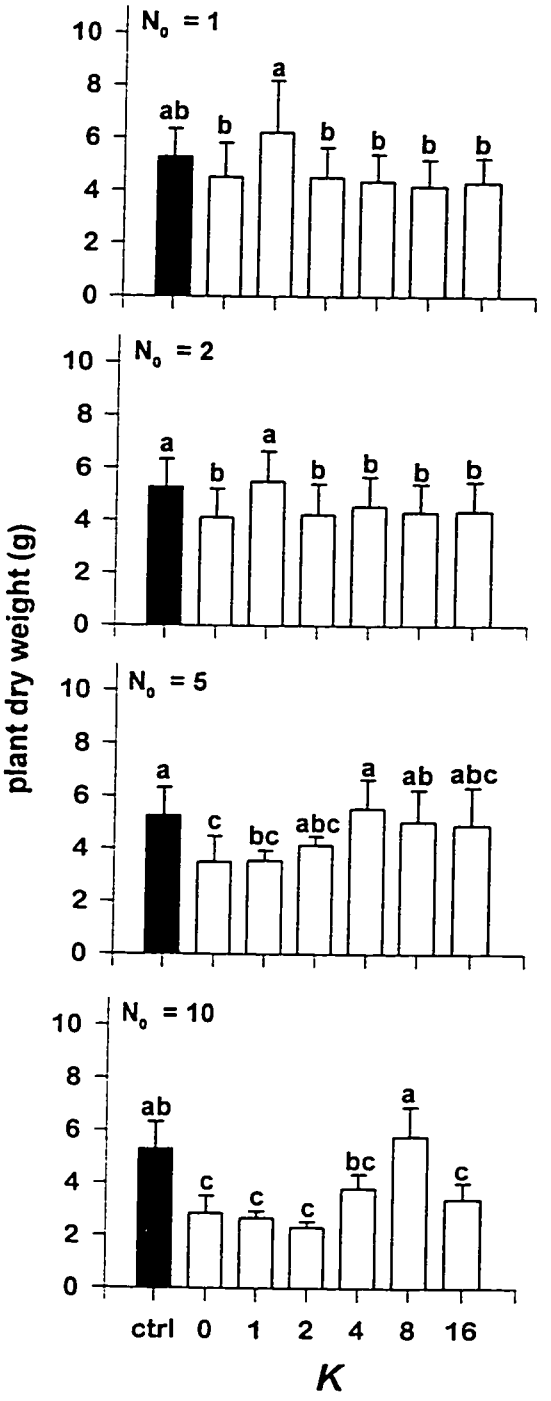

4.25
4.00
3.75
3.50
3.25
3.00
2.75
2.50
log10 plant dry weight (mg)
0
5
10
15
20
25
day
N0 = 2 a
N0 = 5 ab
N0 = 1 abc
N0 = 0 bc
N0 = 10 c
N0 = 1 a
N0 = 0 a
N0 = 2 a
N0 = 5 a
N0 = 10 b
N0 = 0 a
N0 = 1 ab
N0 = 2 bc
N0 = 5 cd
N0 = 10 d
N0 = 0
N0 = 1
N0 = 2
N0 = 5
N0 = 10

Figure 7.9 Biomass at the end of the experiment of sorghum plants inoculated with different initial number of aphids ($N_0$) in relation to the initial number of ladybird larvae (*K*; open bars). Control plants (ctrl; filled bars) are plants with no aphids and no ladybird larvae. Different letters above the bars indicate significant differences between treatments (Waller-Duncan tests, $p = 0.05$). Means and their standard errors are shown.




| | d.f. | MS | F | p |
|---|---|---|---|---|
| Block | 2, 12 | 29.7346 | 54.23 | 0.0001 |
| *K* | 6, 12 | 1.5920 | 2.90 | 0.0549 |

| | d.f. | MS | F | p |
|---|---|---|---|---|
| Block | 2, 12 | 25.6638 | 234.90 | 0.0001 |
| *K* | 6, 12 | 0.8758 | 8.02 | 0.0012 |

| | d.f. | MS | F | p |
|---|---|---|---|---|
| Block | 2, 12 | 18.1275 | 31.67 | 0.0001 |
| *K* | 6, 12 | 2.0458 | 3.57 | 0.0288 |

| | d.f. | MS | F | p |
|---|---|---|---|---|
| Block | 2, 12 | 6.4557 | 8.66 | 0.0047 |
| *K* | 6, 12 | 5.2888 | 7.10 | 0.0021 |

## 7.4 Discussion

### 7.4.1 The ladybirds

The prediction arising from the optimal foraging model of Kindlmann and Dixon (1993) that female ladybirds should refrain from laying more eggs in aphid colonies when the optimum number has been reached is supported here (Fig. 7.2). The additional dimension of increasing the number of aphids available to the ladybirds that was implemented in the present experiment did not, however, result in a drift of the optimum number of ladybird larvae towards higher values, as originally predicted. The canonical analysis revealed that this optimum resides between 4 and 5 initial larvae within the range of aphid colony size experimented, suggesting that the optimum is relatively insensitive to this variable (Fig. 7.3a). This result makes sense since each aphid removed by predation early in the existence of a colony would actually represent several hundreds of individuals a few days later. Thus, the only way a provision of aphids for later during the development of immature ladybirds can be secured is by keeping the initial number of larvae small enough so the exponential growth of the aphid colony is largely maintained. Hence, the relatively modest impact of the ladybird larvae on the final number of aphids when the optimum number is reached (Fig. 7.7), a prediction made by the model of Kindlmann and Dixon (1993).

Although optimal foraging theory predicts that natural selection should act to limit the number of ladybird eggs laid in an aphid colony, the empirical and experimental data do not support this view: adult ladybirds aggregate in areas of high aphid density (Kareiva and Odell, 1987), though their numerical response typically becomes saturated at some aphid density (Mills [1982], *and references therein*), but well after density-dependent cannibalism at the egg-stage (e.g. Mills, 1982) or at the larval/pupal stages (e.g. Osawa, 1993) comes into effect. The discrepancy between the prediction of the optimal foraging model and the actual behavior of female ladybirds, with its consequences in terms of fitness, strongly suggests that female ladybirds cannot precisely assess the quality of an aphid colony. This is not only true for the short-term fate of an aphid colony during its existence, which can be jeopardized by extrinsic factors such as adverse weather (Kindlmann and Dixon, 1993), but is also true for the absolute value of any given colony. Otherwise, the total reproductive numerical response of ladybirds to aphid density would be expected to be roughly the same from location to location, and from year to year, which is not the case. For example, Mills (1982) measured in two non-consecutive years both the reproductive numerical response and the aggregative numerical response of *Adalia bipunctata* (L.) to the density of the aphid *Eucallipterus tiliae* (L.) to give the total reproductive response, or the combined numerical response as he termed it. His results show that while the reproductive numerical response was similar between the two years, the total reproductive

numerical response was 4 times greater at saturation in one year compared to the other, mainly because more adult ladybirds were present in that particular year.

The survivorship data reported here may reveal why ladybird beetles continue to aggregate in areas of high aphid density. Increasing the number of aphids initiating a colony resulted in an increase in the survivorship of the ladybirds at, or below, the optimum number of larvae introduced in the cages (Fig. 7.4). Given this outcome, and the fact that female ladybirds cannot assess the absolute quality of an aphid colony, the best strategy would be to lay more eggs in more dense colonies in any given location and in any given year. One way that female ladybirds can indirectly be *informed* of the relative value of an aphid colony is by a physiological feedback mechanism indicating their state of satiation, as suggested by Kareiva and Odell (1987). This is also supported by the fact that satiated female ladybirds are reluctant to lay eggs when there are no aphids in their vicinity (Evans and Dixon, 1986). Hence, female ladybirds can use this equivalent of a "rule-of-thumb", a combination of their degree of satiation and their rate of encounter with aphids, to rank the value of aphid colonies in a given location. In doing so, they are likely to lay more eggs in colonies with more aphids.

Laying more eggs in colonies with more aphids may very well lead to decreased survivorship for the larvae (Fig. 7.4), with the end result being smaller ladybird populations the following year. When this occurs, however, the aphid

populations have been also drastically decreased in size, as Kindlmann and Dixon (1993) showed using their model, and as evidenced by the experimental results (Fig. 7.7). Inferring from the data here, the consequences at the population level would be that both the ladybird and the aphid populations are reset to their lower densities the following season, but with the accrued benefit of higher survivorship rates for the ladybirds (Fig. 7.4) and decreased predation rates for the aphids (Fig. 7.7) when reproduction is resumed. Consequently, both the ladybird and the aphid populations can build-up over several seasons, until the ladybirds are sufficiently numerous to bring the aphid population to yet another crash and yet another cycle. Such cycles in aphid-ladybird dynamics have been reported numerous times (Hodek, 1970; Dixon, 1985; Heathcote, 1978; Mills, 1982). The ability of ladybird populations to recover from those crashes may be due to the high reproductive rates of aphids.

### 7.4.2 The plant

It was suggested that the sorghum plants would not benefit from what optimal foraging predicts to be the best behavior for female ladybirds since the impact of immature ladybirds on the growth of aphid colonies would be relatively small when the optimum initial number of eggs has been laid. The data, however, point to a different conclusion (Fig. 7.9). Although the match is not perfect, it seems that the reduction in the number of aphids by the ladybird larvae when the

optimum was reached was sufficient for the sorghum plants to achieve similar final size as plants grown in the absence of both the aphids and the ladybird larvae, at least for the treatments in which aphid colonies were initiated with 5 or 10 individuals.

However, it is intriguing to find that, in general, the final sizes of the plants in the treatments in which more ladybird larvae were introduced were smaller than the control plants, those grown in the absence of both the aphids and the ladybird larvae. Nevertheless, these results are somehow consistent with the pattern observed from inoculated plants grown in the absence of ladybird larvae (Fig. 7.8). When followed over the course of the experiment, it appears that the growth of the sorghum plants was stimulated by the presence of a narrow range of aphids. It is thus likely that when there were more ladybird larvae in the cages, they repressed the growth of the aphid colonies beyond the point where the plants would have benefited from the presence of aphids feeding on them.

This subtle balance between the stimulatory feeding of aphids and their otherwise detrimental effect on the growth of the plants was unexpected. Though, Owen and Wiegert (1976) and Owen (1978, 1980) suggested that plants may benefit from aphids feeding on them because these insects are able to synthesize melezitose, a trisaccharide not present in plants and excreted by aphids in their honeydew, which could be a carbon source for free-living nitrogen fixing bacteria,

a hypothesis in part supported by experimental evidence (Petelle, 1980). This process, however, certainly did not operate in the present experiment. First, honeydew was prevented from getting in contact with the growth medium, vermiculite, by the presence of a piece of polystyrene on top of it. Second, the honeydew accumulating on this piece of polystyrene could not be washed into the vermiculite during the watering and feeding of the plants since this was done by filling up the bottom cup. Third, full-strength Hoagland's solution was used to feed the plants, which is likely to have been inhibitory for free-living nitrogen fixing bacteria. Alternative explanations are possible, but they would need to be specifically tested. For example, it is known that several species of aphids are able to synthesize the plant growth hormone indole-3-acetic acid from tryptophan (*see references in* Miles [1987]), but the evidence seems to indicate that the amount produced by individual aphids is too small to stimulate growth. However, growth of blue grama grass, *Bouteloua gracilis*, has been shown to be stimulated by grasshoppers (Dyer and Bokhari, 1976) and Dyer (1980) applied extracts from mouse submaxillary glands directly to sorghum seedlings and found a significant increase in growth compared to control plants.

Compensatory growth as an evolutionary response of plants to herbivory is considered with skepticism (Crawley, 1997). The data presented here indicate that this type of response may very well be overlooked since the observed increase in the growth of the sorghum plants was the result of aphid colonies being of the

right size. If only two types of colonies would have been experimented, say small and large, the pattern found would probably not have emerged.

The narrow range of aphid colony size which was shown to be beneficial for the plants in this experiment makes it unlikely that ladybird beetles can be enlisted as the plants' *extrinsic* defenses (*sensu* Price *et al.*, 1980). Needless to say, these predators cannot be useful in biological control programs against aphids, unless some level of plant resistance is present to prevent the build-up of large aphid colonies and to break the cycles that seem to be typical of the aphid-ladybird interaction.

## — Chapter VIII —

# General Discussion : Natural Enemies as Selective Agents for Plant Defenses

## 8.1 Window of stability: the broader context

Although nearly a century has elapsed since the publication of what is considered to be the first paper on plant-insect interactions (Verschaffelt, 1910), many questions remain unanswered about the role of secondary metabolites of plants in these interactions (Schoonhoven, 1996). Particularly, the diversity and the variation in the levels of these compounds, within and among populations of plants, are still major challenges. Not surprisingly, however, advances in the field are made when complexity, and evolutionary considerations, are addressed directly in investigations of plant-insect interactions (Price, 1991). For example, compelling arguments have been made at a recent symposium of the *Phytochemical Society of America* about the role of redundancy of secondary metabolites in plant defenses (Romeo, Saunders, and Barbosa, 1996). Of particular relevance is the synergism of co-occurring compounds, the lower rate of metabolism of secondary compounds presented as mixtures compared to pure compounds (Berenbaum and Zangerl, 1996), and the lower rate of evolution of counter-adaptations in herbivores when exposed to a mixture of compounds as opposed to an isolated substance (Isman *et al.*, 1996).

It is around this very idea of the durability of a plant defensive strategy that the present research has revolved. The question that was asked was not about why a particular plant species evolved a particular compound conferring protection against an herbivore, but was rather about the variation in the concentration of a particular compound and how natural selection would act to set boundaries to this variation. The argument was made that the natural enemies of a herbivore would be actors in the process of selection, following the proposition of Price *et al.* (1980) that the third trophic level should be considered as the *extrinsic* defense of plants, i.e. participants in the reduction of herbivore density. This proposition was further elaborated in the General Introduction of this thesis (Chapter I) by suggesting that a defensive compound of a plant acts as a valve controlling the amount of energy available to the higher trophic levels in a system. In acting as such, a defensive compound would mediate the interaction between a herbivore and its enemy by acting on the number of consumers available to the predators. The general hypothesis that was formulated was that the durability of a defensive compound would be ensured if variations in concentration actually stabilize the interaction between an herbivore and its enemy, i.e. restrict fluctuations in the number of individuals at both levels over time. With all these considerations in mind, it is in this sense that stability in the whole system is believed to equate durability of a defensive compound.

## 8.2 The working hypothesis

For logistic reasons, a working hypothesis was formulated to test the idea that natural selection acts to constrain variations in the concentration of a plant's defensive compound to a window which favors stability between a herbivore and a natural enemy. Based on a simulation of a predator-prey interaction, it was predicted that, as plant defenses decrease, more prey would be available to the predators, which would result in an increase in their survivorship. There would be a point, however, where more prey, as plant defenses decrease yet further, would not produce accrued benefits to the predators because their functional response eventually saturates. Hence, the point of inflection in a plot of the predator survivorship against prey density would define a window of stability. To the left of that window, i.e., as plant defenses increase, the model also predicts that the interaction between the predator and its prey would be stable, but higher levels of plant defenses may be conducive to an increased rate of counter-adaptation in the prey. To the right of that window, i.e., as plant defenses decreases, the model predicts large variations in the number of both the prey and the predator over time.

### 8.2.1 Preparing for testing the hypothesis in the field

The working hypothesis has been tested in the field in the maize-aphid-ladybird beetle tritrophic system using differentially resistant maize genotypes with DIMBOA-

based resistance, a compound known to be effective against aphids (Long *et al.*, 1977; Beck *et al.*, 1983). In order to test this working hypothesis adequately, data had to be gathered to answer the following questions: 1) How sensitive is DIMBOA to nutrient stress and what is the response of the aphid to the combined effects of plant stress and DIMBOA in the absence of ladybird beetles (Chapter II)?, 2) Are ladybird beetles affected by aphids from DIMBOA-containing genotypes (Chapter III)?, 3) Are ladybird beetles more attracted to some genotypes used in the field in the absence of the aphids (Chapter IV)?

DIMBOA proved to be sensitive to nutrient stress imposed to greenhouse grown plants (Chapter II). However, it is an increase in concentration that was observed, mainly in the high DIMBOA genotype tested, which was already highly resistant to the aphid *R. maidis*. In contrast, plant stress reduced the growth of aphid colonies on a DIMBOA-deficient genotype. Hence, the significant interaction found between plant stress and DIMBOA level on aphid performance, a pattern also observed in the field (Chapter V). In the context of the working hypothesis and the system studied, this heterogeneity brought about at the plant level, in particular with respect to the window of stability, is considered to have a minor influence. However, this conclusion should not be generalized to other systems as plant response to stress is highly variable and may have a destabilizing effect (*see* Gutierrez *et al.*, 1994).

DIMBOA-based resistance did not affect the growth of the ladybird *P. quatuordecimpunctata* fed the aphid *R. maidis* from a high DIMBOA maize genotype. However, a small increase in the duration of the combined pre-pupal and pupal stages was observed when larvae were fed aphids from stressed plants of the high DIMBOA genotype (Chapter III). The implications of this result, along with conclusions from other similar studies, were already fully discussed in Chapter III in terms of intraguild predation and will not be repeated here.

A final precautionary experiment was undertaken before investigating a field system in which the major mediated effect of plant resistance on the interaction between the aphids and the ladybird beetles would be the number of prey available to the predators. This was done by looking at the distribution of predators on stressed and non-stressed plants of different maize genotypes free of aphids to avoid possible indirect effects of plant features, in particular plant color as affected by plant stress, on the foraging behavior of the predators (Chapter IV). The experiment, performed to specifically test for indirect effects, effectively indicated that ladybird beetles were able to discriminate between stressed and non-stressed plants.

Two points should be made here. First, the experiment was implemented using greenhouse grown plants in which variation in color was much wider than what was observed in field grown plants. In other words, the stress treatment

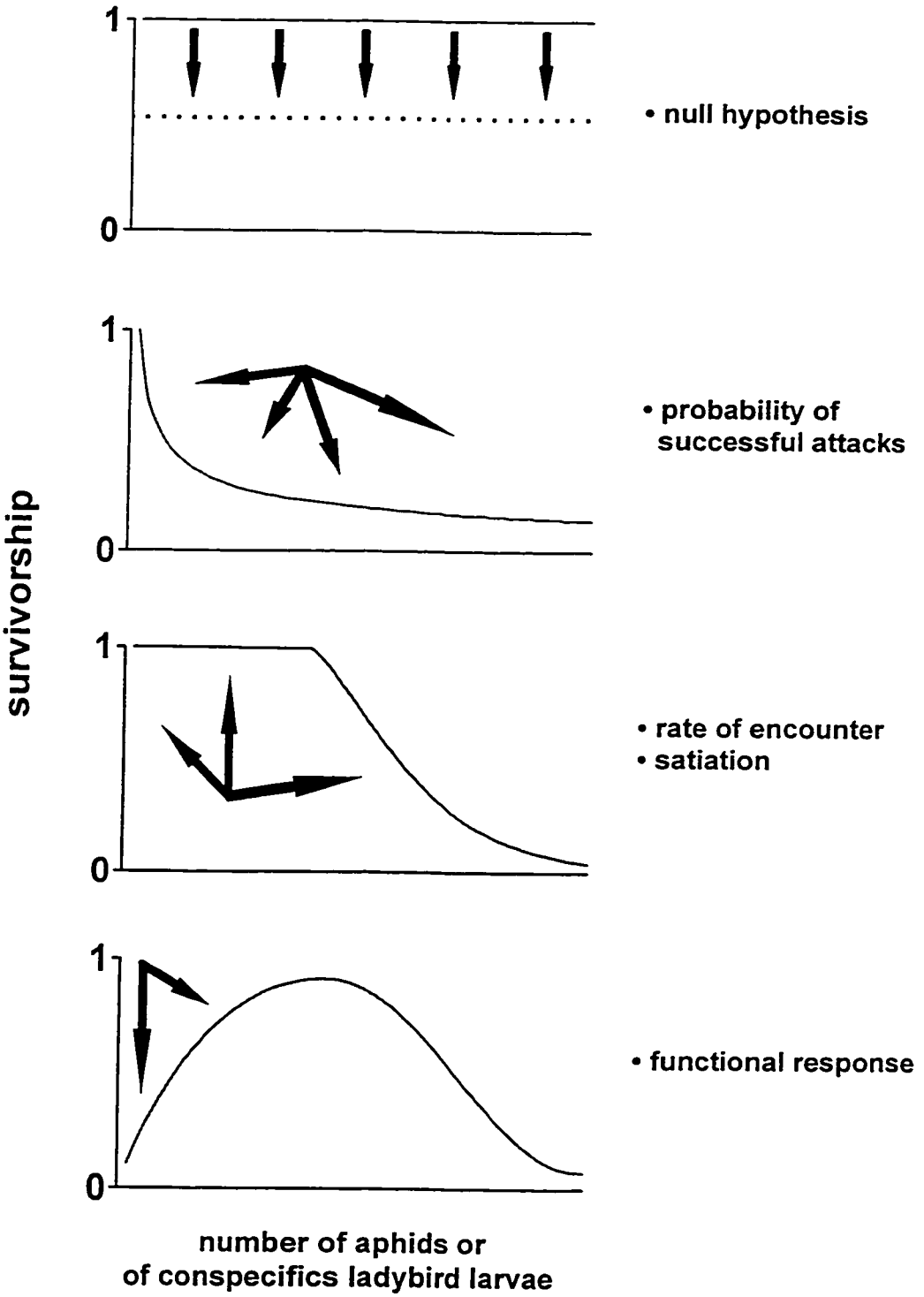
imposed on greenhouse grown plants was much more severe than the stress treatments imposed in the field. Second, plant color should be considered as a factor bearing on the foraging behavior of ladybird beetles, and this point has been discussed in terms of resource partitioning among members of a guild of predators sharing the same prey. Ultimately, it is the presence of aphids, and their abundance, that determine the aggregative and reproductive responses of ladybird beetles (Ives *et al.*, 1993; Kareiva and Odell, 1987; Evans and Dixon, 1986).

### 8.2.2 Putting the working hypothesis to the test

The predicted increase in survivorship of the ladybird larvae as plant resistance decreases and more aphids become available was confirmed in field experiments conducted over three consecutive seasons (Chapter V). Unexpectedly, however, and in contrast to the prediction that survivorship would saturate at higher aphid densities, is the observation that survivorship declined, despite the reproductive response of ladybird beetles tended towards saturation.

Two laboratory experiments were performed to understand the processes underlying the pattern observed in the field (Chapters VI and VII). The results of these experiments are summarized in figure 8.1. The null hypothesis for both experiments was that the survivorship of ladybird larvae was independent of either aphid density or conspecific density and has been rejected in both cases.

Figure 8.1 Summary of factors shaping the survivorship of ladybird larvae.


1
0
• null hypothesis
1
0
• probability of successful attacks
survivorship
1
0
• rate of encounter
• satiation
1
0
• functional response
number of aphids or
of conspecifics ladybird larvae

The first experiment, in which the probability of encounter among conspecific larvae was controlled for, showed that cannibalism occurred readily and was density-dependent in the ladybird *Coleomegilla maculata* (Chapter VI). The model fitted to the data collected in this experiment, a monotonic decelerating curve (Eq. 6.1; Fig. 6.1), indicated undercompensating mortality (Bellows, 1981), in contrast to the overcompensating mortality observed in the field (Fig. 5.9).

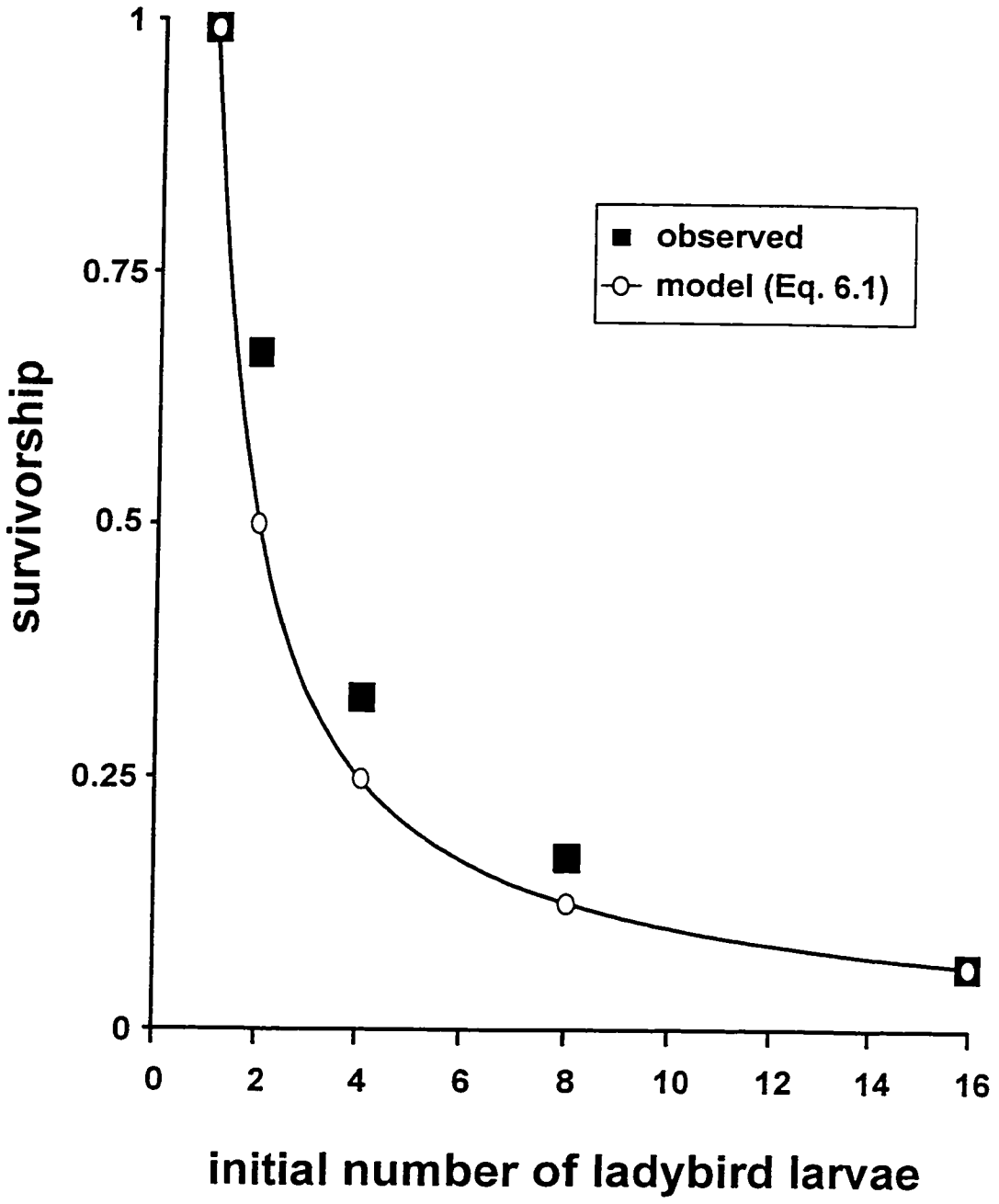
The pattern observed in the laboratory was interpreted as resulting from the increased probability of successful attacks, since the rate of encounter among larvae was controlled for. Cannibalism occurred between larvae of different age, but also between larvae of the same age and, hence, with no large differences in size. It was suggested that cannibalism on larvae of the same size occurred during molting events, as Hodek (1970) reported earlier. I performed a small experiment (*data not shown*) in which previously isolated and starved ladybird larvae of the same age were placed together in a small arena with no food. I observed the behavior of the larvae for *ca.* 1 hour and noted that if larvae were aggressive against each other, none subdued an opponent, lending support for cannibalism occurring on sessile individuals.

In the second experiment performed in the laboratory, larger environments (i.e. caged sorghum plants) were used so to increase the probability of encounter among individuals as the number of larvae increased (Chapter VII). The amount of food available (aphids) to the ladybird larvae was also varied. A comparison is made here between the

observed survivorship of ladybird larvae in cages containing a colony initiated with one aphid (the most severe treatment; Table 7.3) and the survivorship predicted by the model (Eq. 6.1) fitted to the data gathered in the previous experiment (Fig. 8.2). It is assumed that the parameter of the model indicating density-dependence should take a value such as it predicts adequately the survivorship observed at the highest level of initial number of ladybird larvae performed since this level represents the highest probability of encounter among larvae in the experimental cages. In the present case, the value of the density-dependent parameter of the model is such that, irrespective of initial density, only one ladybird larvae survives at the end of the experiment when colonies are initiated with only one aphid. The overall observed and predicted survivorships differed significantly ($n = 5$, $G = 6.90$, $p < 0.05$), suggesting that the rate of encounter effectively varied as initial ladybird density increased in the cages. This result indicates that the environments implemented in the laboratory approached what is likely to occur in the field.

Hence, the effect of increasing encounter rate among ladybird larvae results in the survivorship curve deviating from the predicted curve of a model developed for situations where encounter rate is controlled for (Fig. 8.2). This explains why, up to a certain point, increasing the number of aphids available in the cages resulted in an increase in the survivorship of the ladybird larvae. They were likely to be satiated when an encounter occurred (Fig. 8.1). Past a certain initial density, however, the ladybird larvae depleted the aphid colonies, scramble competition occurred, and the overcompensatory mortality due to cannibalism was apparent.

Figure 8.2 The observed survivorship of ladybird larvae in cages containing one sorghum plant and one aphid colony initiated with one aphid compared to the survivorship predicted by the model (Eq. 6.1).


observed
model (Eq. 6.1)
survivorship
1
0.75
0.5
0.25
0
0
2
4
6
8
10
12
14
16
initial number of ladybird larvae

One last point here, but certainly not a trivial one, is the effect of low prey density on the long term dynamics of the top two trophic levels in a tritrophic system. The range of prey densities below which the functional response of a predator saturates is what is considered as "low" here. It is this portion of the functional response of the ladybird larvae that has been considered in interpreting the increase in survivorship of ladybird larvae (*see* Fig. 8.1) observed in both the field (Chapter V) and in the small environments implemented in the laboratory (Chapter VII). It is also the functional response incorporated in the model by Lundberg and Fryxell (1995) that predicts an increase in the equilibrium density of a predator as the carrying capacity of the prey increases (Fig. 1.2). The overall consequence of the functional response in the low range of prey densities has to be discussed in the light of biological realism, which the great majority of predator-prey models seems to lack. In particular, these models, as the one by Lundberg and Fryxell (1995), incorporates a parameter which defines the conversion rate of food intake into new predator biomass as a constant. I do not wish to discuss here the implications of such reductionism at the scale of one generation, but rather to consider the limitations of incorporating food conversion as a constant in models of predator-prey dynamics. Feeding at less than the maximum rate not only decreases predator survival, but ultimately results in predators being of smaller size (Holling, 1961). Reduced size in itself may be conducive to reduced fecundity in the following generation, but also may lower the capacity of predators to cope with abiotic factors. As far as the conversion rate of food intake being a constant is

concerned, feeding at less than the maximum rate also does not take into account that more energy must be spent by the predators to find their prey when they are scarce. Thus, models which define how many predators will be present in the following generation based on a constant conversion rate predicts increased predator equilibrium as the carrying capacity of their prey increases, whereas biological realism suggests that there may be less predators.

Incorporating biological realism in the interpretation of the field data gathered in the present study strongly suggests, in final analysis, that the most stable situation in a tritrophic system is when prey density is such that predators are satiated. This considerably narrows the window of stability.

### 8.3 Stability in enemy-victim interactions *vs* enemy impact

Conventional wisdom states that for natural enemies of insect herbivores to be a natural selection force acting on the level of plant defenses, some form of synergism should be present between the *intrinsic* and the *extrinsic* defenses of a plant (Simms and Fritz, 1992). In contrast, if natural enemies depress insect herbivore density to very low levels, independently of plant defenses, there will be no selection differential for resistance level in the plant population, or even for resistance at all (Hairston *et al.*, 1960).

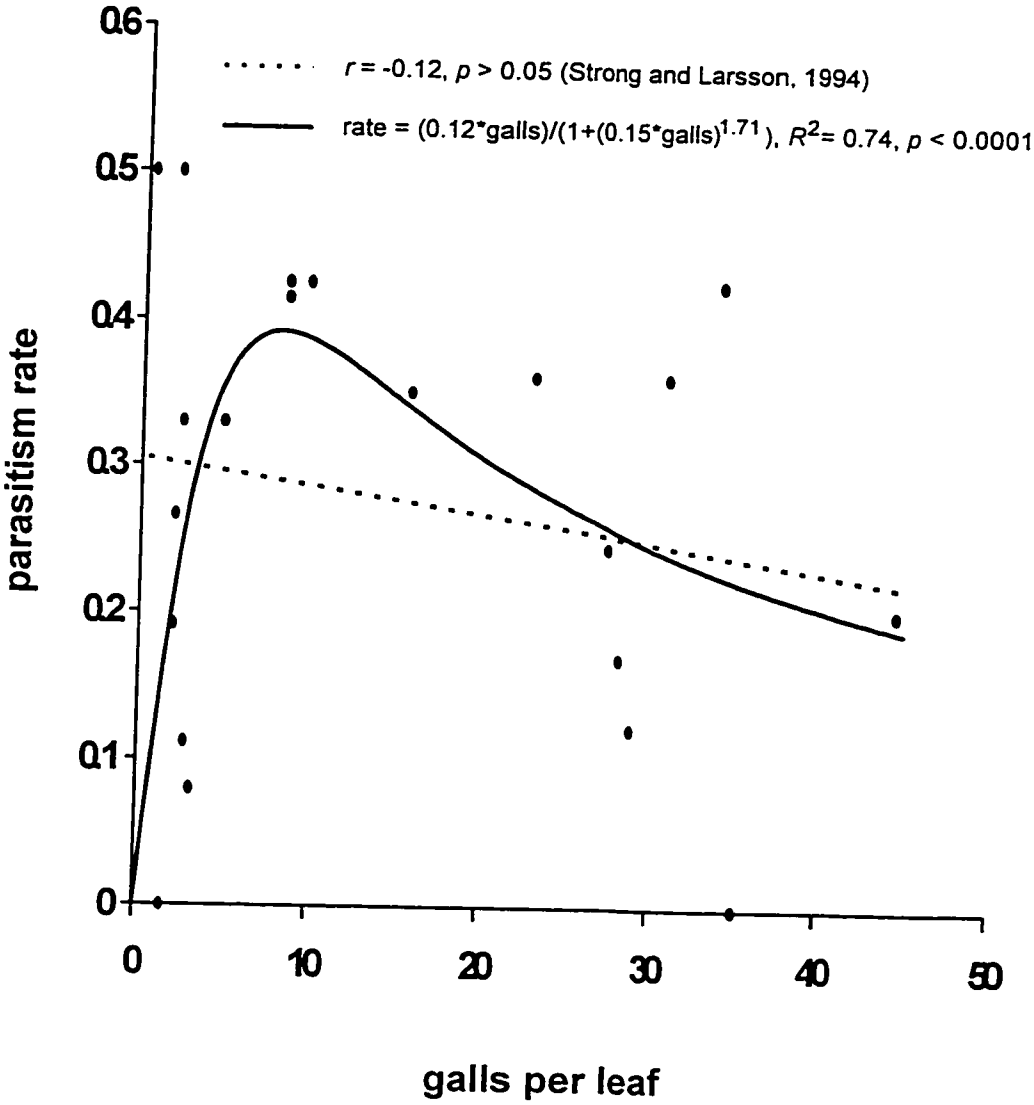
The results of the experiment conducted in the cage environments (Chapter VI) suggest that ladybird beetles can only depress aphid densities when ladybird density is high (Fig. 7.7), confirming the outcomes of a mathematical model by Kindlmann and Dixon (1993). This form of density-dependence is also considered as a form of synergism, but would select for susceptible genotypes in a plant population (Hare, 1992). The results of the present research suggest otherwise, because the reduction of aphid density by ladybird beetles is a transient dynamic. This results from ladybird beetles behaving sub-optimally (Kindlmann and Dixon, 1993). It follows from the cage environment experiment that density-dependent cannibalism in the ladybird population resets ladybird density to low levels in the following generation. This nicely solves the "paradox of enrichment" of Rosenzweig (1971) as it greatly reduces the chances of extinction of the ladybird population.

The point that has been made here is that enemy impact is not the variable of interest *per se* when it comes to test the *Extrinsic Defense Hypothesis* (Price *et al.*, 1980). Unless much is known about the possible long term dynamics of the predator-prey interaction, high enemy impact may simply reflect transient dynamics. In particular, the relevance of plant resistance level in setting a limit to the numerical escape of the prey, by reducing its carrying capacity, makes it likely that the enlistment of a natural enemy as the *extrinsic* defense of the plant should operate to eliminate the most susceptible genotypes in the plant population. This leaves intermediate and highly resistant genotypes in the plant population.

I have so far discussed tritrophic systems in which true predators compose the third trophic level. In the case of parasitoids, for which generation times are similar to their hosts, things are greatly simplified. In particular, in the case of parasitoids, enemy impact is synonymous with reproduction. To my knowledge, there is only one study in the literature that has directly addressed the question of the evolution of plant resistance as influenced by parasitoids (Strong and Larsson, 1994). The study by Strong and Larsson took place in Sweden in a common garden in which several clones of basket willow were grown for a long-term biomass production selection experiment. An epidemic of gall midges occurred, and natural enemies, mainly parasitoids, soon appeared, giving the opportunity to Strong and Larsson to test for differences in enemy impact across willow genotypes.

The results of this semi-natural experiment are reproduced in figure 8.3. The authors concluded that parasitoids had an additive effect to willow resistance because no significant correlation was found between parasitism rate and the number of galls per leaf (Fig 8.3, *dotted line*). However, quite contrary to their original analysis, I found that it was possible to fit a significant non-linear model to their data (Fig 8.3, *solid line*). The non-linear regression explains 74 % of the variation in parasitism rate with respect to gall number and the model indicates that parasitism rate peaks at the low range of gall number. It can thus be argued that this experiment showed evidence that natural enemies can be enlisted as the *extrinsic* defenses of plant as genotypes intermediate in their resistance favored high enemy impact.

Figure 8.3. Parasitism rate in relation to the number of galls per leaf on basket willow grown in a common garden in Sweden. Each data point is from 22 differentially resistant clones. Redrawn from Strong and Larsson (1994; fig. 14.4a). The straight curve (dotted line) is the correlation between the variables which was not found significant by the original authors. The fitted curve (solid line), not present in the original drawing, has been added here and was significant ($y = [d*x] / [1 + [a*x]^b]$; $R^2 = 0.74$, $F_{3, 18} = 17.28$, $p < 0.0001$). The parameter values are given in the figure.


r = -0.12, p > 0.05 (Strong and Larsson, 1994)
rate = (0.12*galls)/(1+(0.15*galls)^1.71), R^2= 0.74, p < 0.0001
parasitism rate
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
galls per leaf

## 8.4 Conclusion

This research has provided evidence that natural enemies can be selective agents for the level of plant defenses. It can be simply argued that susceptible genotypes are likely to be eliminated by selection because of low enemy impact and because of the instability generated by the prey increase being unbounded. At the other extreme, highly resistant genotypes can also be eliminated because counter-adaptations in herbivores can evolve rapidly. Plant populations are thus left with intermediate genotypes in which the range of variation in terms of resistance would depend on the particular parameters of the predator-prey interaction involved.

This thesis offers a new view on the evolution of plant defenses and a conceptual framework for management practices in cultivated areas. By relying on an intermediate resistance strategy, the time necessary for artificial selection programs in crops can be greatly reduced. The durability of resistance mechanisms is also likely to be increased.

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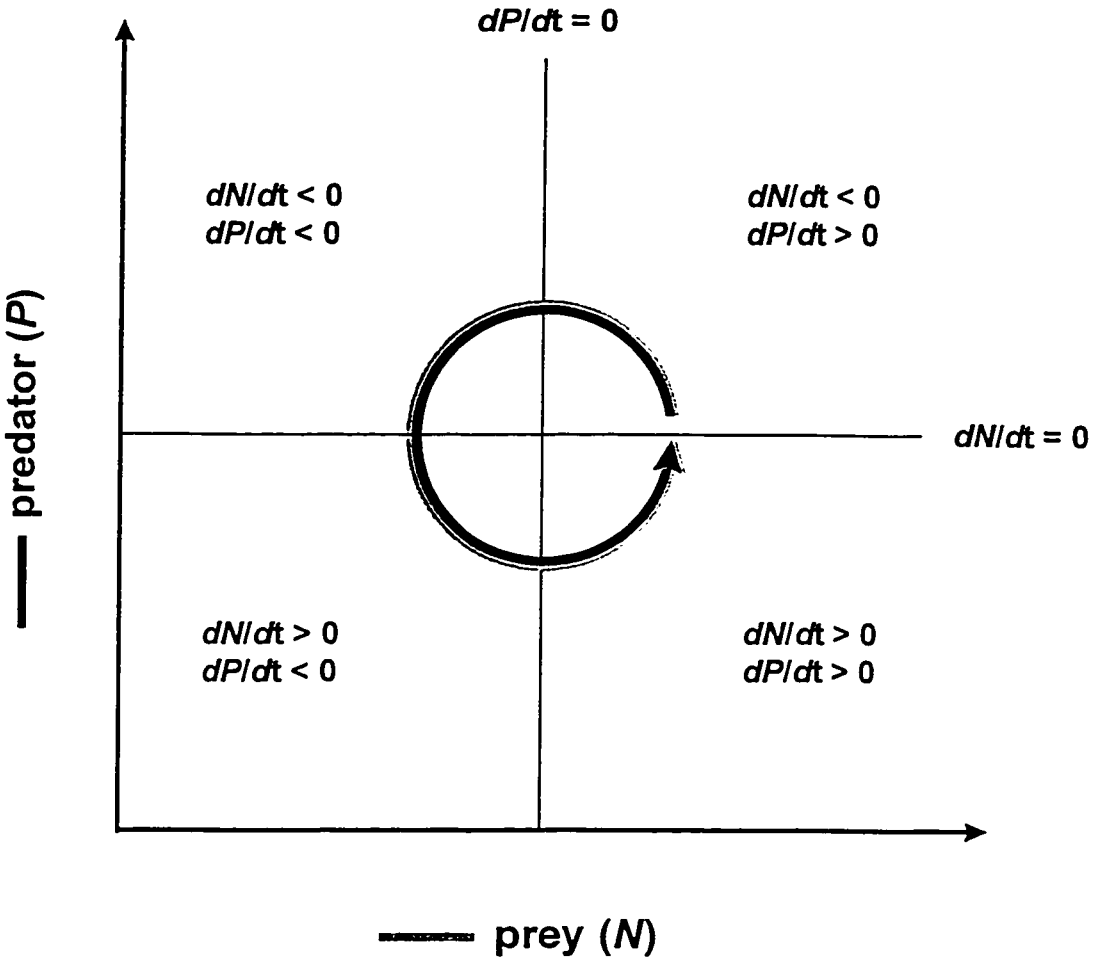
## — APPENDIX I —

### PHASE-PLANE ANALYSIS OF PREDATOR-PREY INTERACTION

Phase-plane analysis was first introduced by Rosenzweig and MacArthur (1963) as a means to interpret graphically the dynamics of a predator-prey interaction. The time factor is implicit in this representation of the interaction in which coupled predator-prey densities are plotted at any point in time of the interaction. Variations in the density of either the prey or the predator are interpreted in relation to their respective isoclines. An isocline is a line composed of all the coupled predator-prey densities for which no increase or decrease would be observed in the predator or in the prey populations. In the basic Lotka-Volterra model, there is no self-limitation in either the prey or the predator populations. In this model, the per capita kill rate of the prey by the predator (*i.e.* its functional response) is a linear function of prey density. These two assumptions of the basic model yield a horizontal isocline for the prey and a vertical isocline for the predator (Fig A1.1). Above the prey isocline ($dN/dt = 0$), there is many predators, and the prey population will decline. Below the prey isocline, there are too few predators, and the prey population will increase. At the left of the predator isocline ($dP/dt = 0$), there are too few prey, and the predator population will decline. At the right of the predator isocline, there is many prey, and the predator population will increase.

Figure A1.1. Phase-plane diagram of a predator-prey interaction derived from the basic Lotka-Volterra model.


dP/dt = 0
dN/dt < 0
dP/dt < 0
dN/dt < 0
dP/dt > 0
dN/dt = 0
dN/dt > 0
dP/dt < 0
dN/dt > 0
dP/dt > 0
predator (P)
prey (N)

Lundberg and Fryxell (1995) modified the assumptions of the basic Lotka-Volterra model to make it more realistic. They first included self-limitation in the prey population, that is, the prey does not reproduce as fast when its density approaches carrying capacity as when there are few individuals. They also modified the kill rate of the predator as to set a limit to the number of prey killed per predator per unit of time. This produces saturating functional and numerical responses for the predator in relation to prey density (Type II responses; Holling, 1959).

Lundberg and Fryxell (1995) have developed the following equations to describe the dynamics of the prey:

$$dN/dt = rN(1 - N/K) - (aNP / (1 + ahN))$$

and of the predator:

$$dP/dt = P((caN / (1 + ahN)) - d)^{\dagger}$$

with $N$ and $P$, the respective densities of the prey and the predator; $r$, the intrinsic rate of increase of the prey; K, the carrying capacity of the prey; $a$, the attack rate of the predator; $h$, the time spent by a predator to handle one prey; $c$, the conversion rate of consumed prey into new predators; and $d$, the density-independent predator mortality rate.

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† Note that the equation for the predator in Lundberg and Fryxell (1995) was: $dP/dt = P((caNP / (1 + ahN)) - d)$. The second $P$ term on the right-hand side is obviously a typo.

The prey isocline is found by solving:

$$dN/dt = rN\,(1 - N/K) - (aNP / (1 + ahN)) = 0$$

which gives:

$$P^* = (r/a)\,(1 - N/K)\,(1 + ahN)$$

The predator isocline is found by solving:

$$dP/dt = P\,((caN / (1 + ahN)) - d) = 0$$

for which either:

$P = 0$ (i.e. when there is no predator, the predator population cannot grow)
or

$$(caN / (1 + ahN)) - d = 0$$

which gives:

$$N^* = d / (a\,(c - dh)).$$

## — APPENDIX II —

### THE PREDATOR RATE OF INCREASE

In order to make testable predictions about the effect of increasing the carrying capacity of a prey on the stability of a predator-prey interaction, it is necessary to know how prey density will affect the growth rate of the predator population. Here, I derive the rate of increase of the predator population as affected by the functional response included in the model of Lundberg and Fryxell (1995).

Lundberg and Fryxell (1995) gave the following equation for the predator, which includes a Type II functional response to prey density:

$$dP/dt = P\,((caN / (1 + ahN)) - d)$$

where $N$ and $P$ are the respective densities of the prey and the predator, $a$ is the attack rate of the predator, $h$ is the time spent by a predator to handle one prey, $c$ is the conversion rate of consumed prey into new predators, and $d$ is the density-independent predator mortality rate.

We can simplify this equation by writing:

$dP/dt = sP$

where $s$ is the rate of increase of the predator. From this, we thus know that:

$s = cy - d$

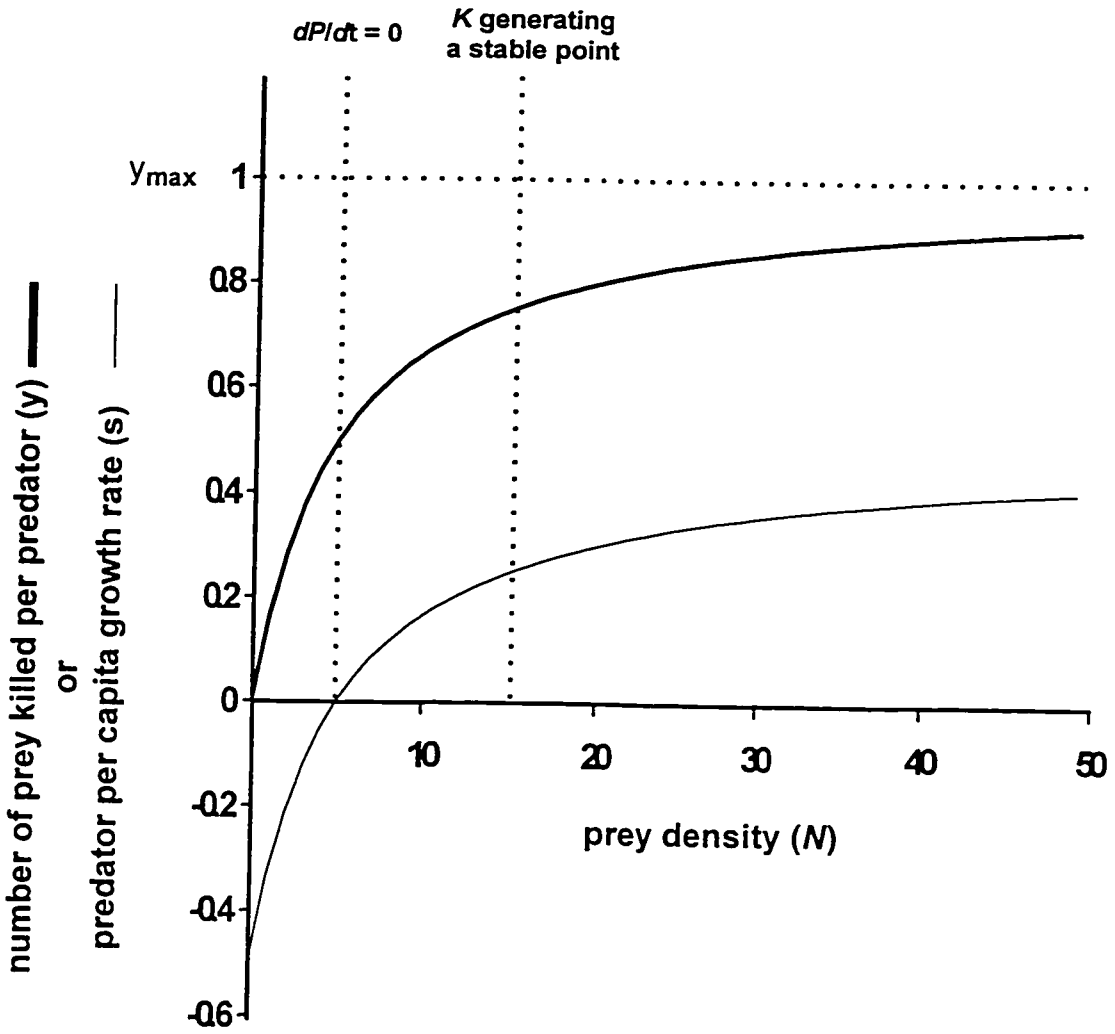
where y is the number of prey killed per predator (*i.e.* the functional response), which is given by:

$y = ( aN / ( 1 + ahN ))$

for which $y_{max}$ will be asymptotically approached when $N \rightarrow \infty$ and will be determined by $1/h$ (Fig. A2.1).

For the parameter values indicated in the legend of figure A2.1, we know that the predator will exhibit positive growth ($s > 0$) when prey density will have reached a critical value. This critical value determines the location of the predator isocline drawn in figure 1.2. Because the predator functional response (y) saturates, we find that its growth rate ($s$) also saturates.

Figure A2.1. The functional response of the predator (y) and its per capita growth rate ($s$) in relation to prey density. The maximum number of prey killed per predator is $y_{max}$ (horizontal dotted line). The predator isocline shown in figure 1.2 is indicated by the first vertical dotted line ($dN/dt = 0$). The second vertical dotted line indicates the location of the prey's carrying capacity ($K$) which produces a stable point as predicted by the model of Lundberg and Fryxell (1995; *see figure 1.2c*). Functional response: $y = ( aN / ( 1 + ahN ))$; $a = 0.2$, $h = 1$). Predator rate of increase: $s = cy - d$; $c = 1$; $d = 0.5$.


dP/dt = 0
K generating
a stable point
ymax
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
10
20
30
40
50
prey density (N)
number of prey killed per predator (y)
or
predator per capita growth rate (s)

Because the growth rate of the predator saturates, there is not much accrued benefit from increasing prey density. The carrying capacity ($K$) of the prey which yields a stable point for the interaction (*see* figure 1.2.c) has been drawn in figure A2.1. Past this prey density, the growth rate of the predator continues to increase, but at a decelerating rate.

Thus, this treatment of the growth of the predator population in relation to prey density indicates that the survivorship of the predator will increase as prey density increases, but at a decelerating rate. Hence, collecting data to compute the survivorship of immature stages of a predator in relation to prey density as mediated by plant resistance should yield a curve similar to the one presented in figure A2.1 for the predator rate of increase. The window of stability of the interaction would then be defined by the range of prey density over which the survivorship of the immature stages of a predator starts to saturate.

## — APPENDIX III —

## DETERMINATION OF DIMBOA CONCENTRATION IN LATE-WHORL AND TASSEL TISSUES OF MAIZE

DIMBOA was extracted from 0.50 g of freeze-dried samples of late-whorl and tassel tissues in 4 times 10.0 ml of 70 % methanol using a polytron mixer set at high (1 min. per extraction). After each extraction, the samples were centrifuged for 5 min. at 1500 r.p.m.. Methanol in the pooled supernatants was evaporated using a rotary evaporator with the water bath set at 40 °C. The pH of the residual water fraction was lowered to 2.0 with the addition of 1N HCl. The water fraction was then extracted 3 times with 10.0 ml of ethyl acetate. The pooled extracts were evaporated to dryness as before and kept at -20 °C until analysis.

Stored samples were suspended in 2.0 ml of 50 % methanol and sonicated for 5 min. Samples were then centrifuged for 5 min. at 2,000 g and the supernatants were filtered through a 0.2 μm nylon membrane before analysis on a HPLC. The HPLC analyses were performed on a Beckman Gold system equipped with a 502 auto-sampler, a 126 binary pump, and a 168 diode array detector with two channels open (263 nm for maximum resolution of DIMBOA and 295 nm for maximum resolution of MBOA, the degradation product of DIMBOA). The solvent system was methanol (A) and 10 mM $H_3PO_4$ (B). Solvents were run at a flow rate

of 1.0 ml/min. using the following linear gradients: 15-55 % of A in B in 15 min., 55-80 % of A in B in 5 min., 80-100 % of A in B in 2 min., 100 % of A held for 8 min., 100-15 % of A in B in 2 min., and 15 % of A held for 3 min. The samples were injected into a Beckman RPC18-ODS (5 μm particle size), 250 x 4.6 mm, column using a 20 μl injection loop.

Concentrations were calculated from standard curves obtained from pure reference compounds. DIMBOA was synthesized by Atkinson *et al.* (1991) and MBOA was purchased from Sigma. MBOA was not detected in the samples.

# IMAGE EVALUATION
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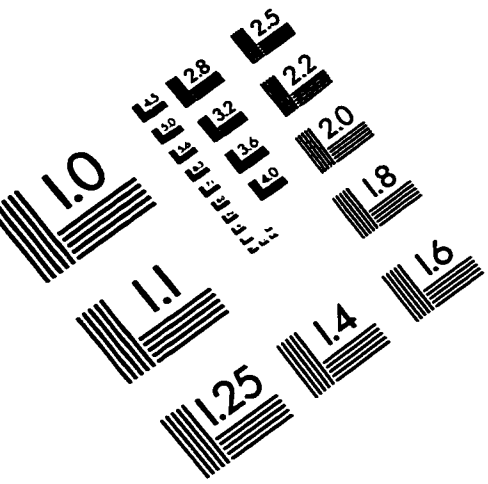


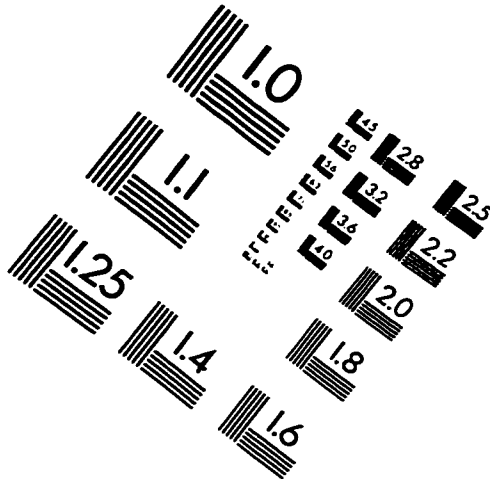


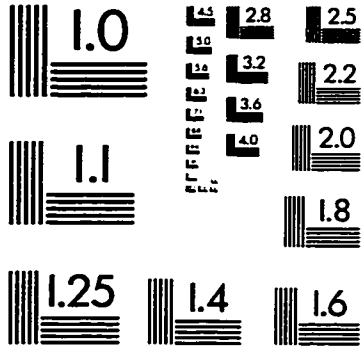


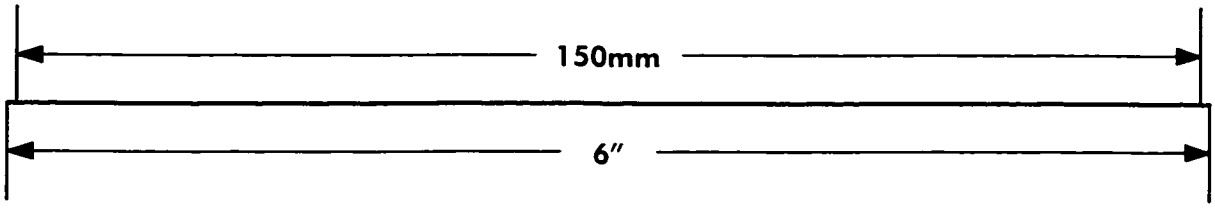


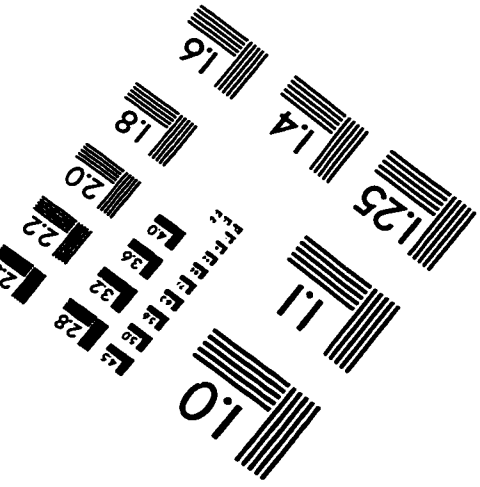


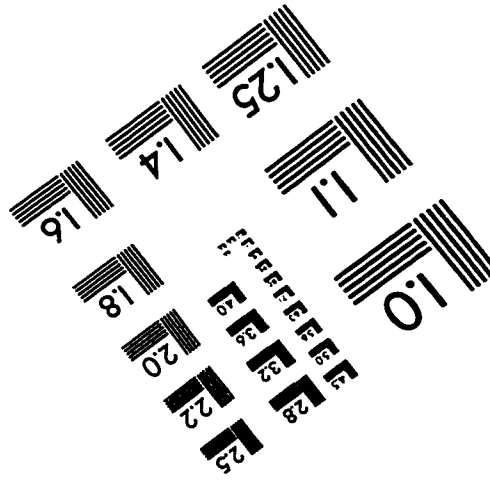


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