SESSION 3
FIELD EXPERIENCES

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Factors affecting the impact of semiochemicals in insect pest-management: a simulation model of an attracticide strategy

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ABSTRACT

Biocontrol techniques based on behavioural manipulation of pest insects and/or their associated natural enemies depend on behavioural and density dependent processes and are thus more complex and dynamic compared to insecticide applications. One way to understand the factors influencing success or failure of the field performance of these novel techniques is to simulate the dynamics in a computer model. In this paper we present a simulation model that was developed to study factors affecting the efficacy of a pheromone-based attracticide product for control of the codling moth, *Cydia pomonella*. Abiotic factors, such as spacing and durability of the attracticide formulation, as well as biotic factors, such as population biology and behavioural characteristics of the pest species, were investigated. The model is constructed in such a way that it can be used to predict the feasibility of attract and kill for different pest/crop systems. Factors affecting the possibilities and constraints of the attracticide strategy as a pest control method are discussed.

INTRODUCTION

Crop protection is usually achieved by poisoning the pest with a pesticide, but it can also be achieved by manipulating the behaviour of insects. Chemical stimuli play an important role in the behaviour of insects, such as in their search for food, a sexual partner, and a habitat or a host for their progeny. Chemicals involved in interactions between organisms are called semiochemicals (Nordlund & Lewis, 1976; Dicke & Sabelis, 1988). Semiochemicals are divided into pheromones, which mediate interactions between organisms of the same species (e.g. sex pheromone, alarm pheromone), and allelochemicals, which mediate interspecific interactions (e.g. flower volatiles used to attract pollinators). Semiochemicals that attract or repel insects have the potential to be used in insect pest management. Semiochemical-based pest control can be employed in a variety of ways: (1) by directly affecting the target pest, such as in mating disruption, mass trapping, or the use of oviposition repellents or feeding deterrents, and (2) indirectly, by affecting host or natural enemies of the pest insect such as by inducing defence behaviour of plants or by attracting natural enemies of the pest (Bottrell *et al.*, 1998; Agelopoulos *et al.*, 1999).

Biocontrol techniques based on behavioural manipulation of pest insects and/or their associated natural enemies depend on behavioural and density dependent processes and are thus more complex and dynamic compared to insecticide applications. For instance, in sex pheromone based mating disruption and attracticide control techniques, only adult males are affected. This makes the system very sensitive as every small deficiency in the male control system has a major impact on the efficacy of these control methods. One way to understand the factors influencing success or failure of the field performance of these novel

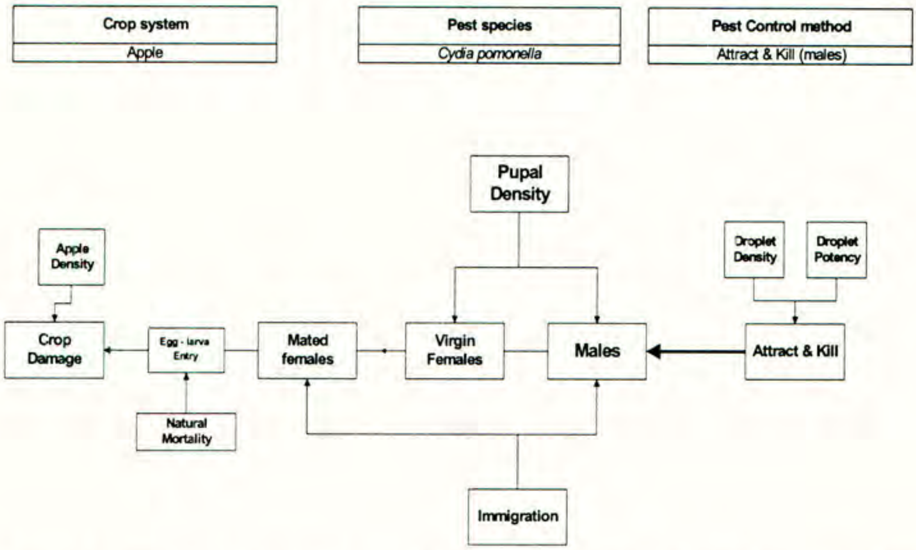

Figure 1. Overview of lure and kill model structure. The simulation model is constructed with parameters confined in: (1) Orchard environment (2) *Cydia pomonella* dynamics (3) attracticide application characteristics.

techniques is to simulate the dynamics in a computer model. Recent examples of simulation models for biocontrol methods are Cooke & Régnière (1996) for *Bacillus thuringiensis* efficacy against the spruce budworm and Roermond *et al.* (1997) for parasitoid efficacy (*Encarsia formosa)* against the whitefly. In this paper we present a simulation model for population control of pest insects using a pheromone-based attracticide, with a special emphasis on the codling moth. The codling moth, *Cydia pomonella* (Lepidoptera), is a pest of world wide importance in commercial pome fruits. The use of mating disruption, based on the premise that male moths are unable to locate calling females in an environment permeated with sex pheromone, has become more popular as a means of controlling the codling moth (Minks & Cardé, (1995); Witzgall & Arn, (1997)). However, the method has several drawbacks, restricting the employment of mating disruption (Minks, 1997) and several alternative strategies for semiochemical use are being sought. In this respect, the lure and kill strategy represents an interesting biorational approach to the control of pest insects and has in various different forms recently be used successfully to control the codling moth, an important pest in pome fruits (Hofer and Brassel, 1992; Charmillot et al 1997 Lösel et al. 2000). The use of a formulation containing an insecticidal agent obviates the need for costly, cumbersome physical traps and allows the necessary density of killing point sources needed to compete effectively with the attraction of the natural sex-pheromone source, the "calling" female insect. Point source formulations have been described for the control of *Anthonomus grandis* (McKibben *et al.*, 1990), *Pectinophora gossypiella* (Hofer & Brassel, 1992), *Cydia pomonella* (Hofer & Brassel (1992), Charmillot et al. (1996), Lösel *et al.* (2000)) and *Rhagoletis* spp. (Liburd *et al.*, 1999). The aim of the work described in this paper is to assess abiotic factors (e.g. attractant release characteristics, spacing and durability of the attracticide formulation,) which might, under field conditions, limit the reliability of an attracticide over the duration of the season and to investigate biotic factors such as the population biology and behavioural characteristics of the pest species which may influence the efficacy of the strategy. For more details on the application of the attracticide we refer to Lösel et al. (2000).

MODEL OUTLINE

The model is an extension of the model presented by Roelofs *et al.* (1970). In figure 1 an overview is given of the structure of the model used to simulate the control efficacy of an attracticide against the codling moth. Parameters of the crop system serve as the basis for the determination of the economic threshold level. These include fruit density and natural mortality factors acting on egg and larval stages of the codling moth. Pest input-parameters include pupal density, daily survival rate and immigration rate. Key model state variables include male moth density, virgin female density and mated female density. The cumulative number of mated females determines, in combination with the crop system parameters, the level of expected damage (i.e. larval infested apples). The number of virgin calling (i.e. pheromone releasing) females determines the level of competition with the attracticide sources. Parameters of the attracticide application include the droplet density and droplet potency. Droplet potency is a combination of relative attractiveness of the pheromone component and relative knockdown potential of the insecticide component. Maximum attractiveness of the synthetic pheromone is optimised to give a nominal value of 1, which equals attraction towards a calling female. In sex pheromone systems it usually not possible to develop an attractant which more attractive than the natural source (i.e. a calling female). For the codling moth we found a distinctive pheromone dose-response curve (Lösel *et al.*, 2000). Droplet potency decreases with exposure time to ambient weather conditions (Lösel *et al.,* in prep.). This was incorporated in a degradability rate parameter (default value: 0.025 day^{-1}).

Population density and phenology

The model keeps track of the number of male and female moths for throughout a season. The fraction of moths emerging from the pupal population on a particular day is calculated from a realistic flight curve at a particular site (estimated from *C. pomonella* trap catches throughout the season). As a default, the flight curve of *C. pomonella* at Höfchen (1995) was used, which had two distinct peaks (i.e. generations) (Figure 2). An estimation of the total pupal density per ha was taken from data of mass trapping experiments in the literature and from previous field experiments (unpublished data). For *C. pomonella,* the mean moth density/ha is estimated to be 1000-2000 moths/ha. The total number of males/ha on a particular day is the sum of the number of males emerging that day, the number of males surviving the previous day and the number of males immigrating from neighbouring plots:

$$M_i = (1-SR) * NM_i + (F_{i-1}) \cdot d + Im * NM_i$$

where: M_i is the total number of males/ha on day i
SR is the sex ratio of the emerging animals (fraction females)
NM_i is the number of emerging males on day i
F_{i-1} is the number of matings on the previous day
d is the adult survival rate (i.e. fraction surviving another day)
Im is the immigration factor expressed as fraction of density

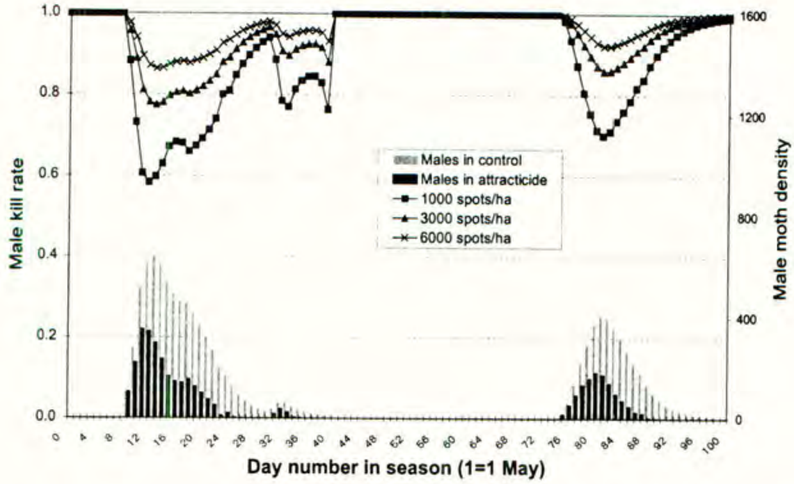

Figure 2. Predicted attracticide-caused mortality throughout a typical season. Model parameters: Attracticide application dates day 1 and day 74, pupal density 2000/ha, grey bars indicate number of males without attracticide control, black bars indicate number of males in plot with 3000 attracticide spots. Flight curve based on Hofchen 1995. Moth survival rate 0.75. Lines at top of graph indicate potential male kill rate per day as a function of relative attracticide potency in relation to female density at 1000, 3000 and 6000 attracticide spots per ha.

Probability of mating

The key factor to the success of a pheromone-based attracticide approach is a significant reduction in the number of matings taking place. The probability that a male mates with a female is dependent on the number of attracticide spots and their relative attractivity compared to a calling female. In the model, the probability of mating in the absence of competition from attracticide spots is set at 1. Thus the probability of mating decreases with a high density of attracticide spots and a high relative attractiveness per spot:

$$P_i = V_i / A_i * N + V_i$$

where: P_i is the probability of a male to mate with a female on day i.

Vi is the total number of virgin females/ha on day i.

Ai is the relative attractiveness of the formulation on day i.

N is the number of attracticide spots/ha.

The probability of mating (P_i) is not constant throughout the season because the attractiveness of the attracticide spots (Ai) wanes with time following application as a consequence of weathering. In the model, the probability of mating is calculated for each day incorporating the attractivity of the attracticide spots on that day. Because the model assumes that a male is either attracted to an attracticide spot or to a calling female the number of males removed per day is thus:

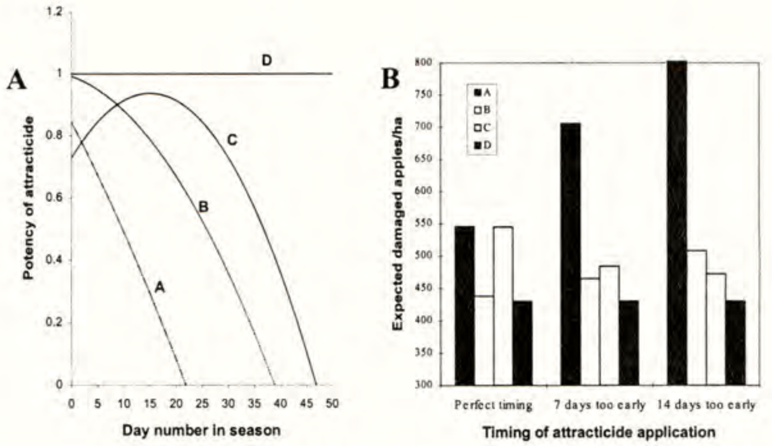

Figure 3 (A) Degradability rate of different formulation types through the season applied at day number 0. Indicated are relative attractiveness of formulations on a particular day in season. The maximum attractivity of 1 equals the attractiveness of a wild calling female.(B) Predicted control efficacy of formulation types (a,b,c,d) with different degradability rates (see figure 3a) applied at different application dates. Bars indicate predicted damage, horizontal line indicates economic threshold (1%.). Perfect timing set at one day before start of flight. Main simulation model parameters: *C. pomonella* pupae/ha: 6000, Number of attracticide sources/ha: 3000, trees/ha: 1000, apples/tree: 175, entries/female: 3, Flight curve: Höfchen 1995.

$AK_i = 1\text{-}P_i * M_i$

where: AK_i is the number of males killed on day i.

$1\text{-}P_i$ is the probability of a male to be attracted and killed by an attracticide spot on day i.

M_i is the total number of males/ha on day i.

The formula of the probability of mating predicts that a complete eradication of the male population is unlikely and thus with increasing moth density some matings will take place. Thus the number of matings not only depends on the number and attractiveness of the attracticide spots, but the efficacy of the attracticide also depends on the moth density. If the density of moths increases the number of matings (and thus expected damage) increase. This density effect can be compensated by increasing the number of attracticide spots per tree. This density dependent effect can also be seen within a season (Figure 2). The kill rate (i.e. percentage of males removed from the population) of the attracticide spots is density dependent. When the density of moths is at its peak the kill rate drops (Figure 2). This density dependent effect can be diminished by increasing the number of attracticide spots. The density dependent effect on attracticide efficacy can also be made clear at tree level. The probability that a male approaching a tree with a calling female will successfully mate will decrease with an increasing number of attracticide spots in the tree, but increase with an increase in the number of calling females in the tree.

Durability of attracticide

To simulate the efficacy of attracticide formulations with different degradability rates, model runs were made with four different patterns based on variation of attraction through time. These patterns were tested in relation to three different application dates within to the flight curve. Using data from experimental formulations, regression curves were calculated for the relation between time since application and relative potency of the attracticide formulation. As control a theoretical formulation was used with no decrease in attraction through time. The regression curves used for the simulations are shown in Figure 3a. The predicted efficacy of the four formulation types was simulated at three different application dates (Figure 3b). The simulation results show that the theoretical formulation (D in figure 3a,b) has no decrease in attractiveness through time and its efficacy is thus independent of the application date. The formulation with the fastest loss in attractiveness through time (A) is very sensitive to the timing of the application and this formulation can hold the damage below the economic threshold only when it is perfectly timed. The slow release formulation (C) has its optimal attraction 2-3 weeks after application. The efficacy of this formulation is thus lower when it is perfectly timed, compared to the applications 7-14 days too early. However, the time window in which this slow release formulation is active is the longest for the three real formulations. This makes this type of formulation ideal for situations with unpredictable flight curves and patterns.

FACTORS AFFECTING THE IMPACT OF SEMIOCHEMICALS

Ecology of the target species

Despite the fact that the presented model ignores relevant factors such as the spatial structure of a population or the range of attraction of the single attracticide sources, it increases the explanatory insight in the lure and kill dynamics. As demonstrated with the presented model, the level of competion with natural semiochemical sources is an important factor determining the efficiency of a attracticide based control method. Pest species with a moderate population density, such as the codling moth (1500 moths /ha), can be controlled as long as the number of attracticide sources is several factors higher than the number of natural sources (calling female moths). Pests species with high population densities, as for example seed weevils in oilseed rape, which can reach densities of 1-2 million weevils /ha (Hokkanen *et al.*, 1986), can not practically be controlled with a pheromone-based attracticide.

The feasibility of a semiochemical-based control method can also be determined by the type of semiochemical. In this respect, aggregation pheromones are highly favourable semiochemicals to incorporate in an attracticide device. Aggregation pheromones are distinctive from background odours, usually have a long range attraction on both males and females. Their ability to attract females make aggregation pheromones well suited for an attracticide strategy. Furthermore, the number of sources where natural aggregation pheromone is produced is relatively low due to the clumped distribution of calling individuals, hence the competitiveness of the attracticide sources with natural sources is high. Attracticide devices are used successfully against the cotton boll weevil and bark beetles (Foster & Harris, 1997 for references).

The addition of host-plant volatiles (i.e. kairomones for foraging females) to an attracticide formulation based on sex pheromone attraction could increase the impact of an attracticide on population reduction. For several moth species, it has been demonstrated that male response to sex pheromone is enhanced in the presence of host-plant volatiles (Landolt & Phillips, 1997). However, a significant reduction in the expected damage could be achieved if both males and mated females are removed from the population. Many insects use specific host-plant volatiles in their search for suitable oviposition sites. Female *C. pomonella* deposit their eggs near (young) apples and they probably use specific apple volatiles to locate the fruits. One of the problems of kairomone impact is that the number of natural hosts the attracticide point sources have to compete with can be very high. For instance, to attract and kill mated females in search of an oviposition site the 3000 attracticide drops (per ha) have to compete with 100,000 apples (per ha). To have any impact on the female population the relative attractiveness of the formulation should be several orders of magnitude higher than the natural sources ($A_0>>1$). A volatile stimulus that is ubiquitous in the environment can only be successful for manipulating behaviour if by virtue of its intensity or quality can be perceived by the insect above the background level of that stimulus. For several systems, this could be achieved, but the only disadvantage is that the concentration of the kairomone has to be very high, which makes the development of a durable bait difficult (i.e. although $A_0>>1$, degradability/time (Ac) is high).

The level of dispersal can be a crucial factor determining the feasibility of a semiochemical based control method. For example, females of the European cornborer (*Ostrinia nubilalis*) call and mate in wild grass habitats and move after mating to maize fields (DeRozari *et al.*, 1977). This is probably the main reason why mating disruption in maize fields does not work for *O. nubilalis* control.

Agroecosystem characteristics

Whether a semiochemical-based control product can be successfully employed can depend on the crop characteristics. For a successful mating disruption with volatile pheromones it is important that there is a constant pheromone cloud within the crop canopy (Karg & Sauer, 1995).) The volatility of the pheromone is determined by the air currents within the crop, which depend on plant architecture and the slope of the cropping area. In this respect it is interesting to note that mating disruption trials in protected environments, such as tomato and sweet pepper cultures in greenhouses, were highly successful (Van der Pers & Minks, 1998). The prerequisite of a constant pheromone cloud within the crop environment may constrain the employment of mating disruption in small annual crops in the open field.

Product formulation

The nature of the formulation's release characteristics is paramount in determining the efficacy of the formulation. For example, an attracticide formulation not only should protect the pheromone and insecticide components from UV-induced degradation, but also enable a controlled release of pheromone and enable optimal take up of the insecticide. One of the important factors determining the attracticide efficacy is a proper timing of the application. Due to the degradation of the attracticide formulation in time the control effect may be too low if the moth flight is long (3-4 weeks). Among the factors that determine the durability of the attracticide are the surface type (e.g. spot size and absorption rate on bark, leaf), temperature driven evaporation of pheromone and ultraviolet driven degradation of pheromone and insecticide.

Evolution of behavioural resistance

Repeated behavioural manipulation (e.g. mating disruption with a synthetic pheromone) might result in the selection of resistant populations, similar to the development of resistant strains following the repeated use of insecticides. Evolution of behavioural resistance requires that there is genetic variation in response to an attractant and a genetically based decrease in response to the attractant increases the fitness of the pest (Gould, 1991). Although there is evidence that there is genetic variation in pheromone release and response (McNeil, 1992), there are to our knowledge no reported cases of populations that are resistant to a particular semiochemical-based product. One of the reasons for the lack of resistance may be that there is generally a lack of evolutionary flexibility for the response to a semiochemical. If the response to a particular semiochemical significantly helps the insect to locate a mate, a food source or an oviposition site, a decrease in response compromises fitness.

Interspecific interactions

An advantage of a pheromone based attracticide product is it species specificity. Despite the fact that a broad spectrum insecticide is used as the killing agent in the formulation, the point source application and species specific attraction warrants that beneficial insects such as natural enemies are not affected. Thus attracticide products are well suited to be incorporated in integrated pest management strategies.

The species specificity of pheromone based products can also have disadvantages. In contrast to insecticide sprays, secondary pests are not affected. An economic solution could be the development of a co-formulation incorporating the pheromone systems of the target species. In this way one attracticide application could affect two target species simultaneously. However, there are several constraints for the development of multispecies attracticide. In our *C. pomonella* system the summerfruit tortrix moth *Adoxophyes orana* is an important secondary pest. Given that the durability of reliable attracticide product is a few weeks it is important that the flight phenology of the target species overlap. In most years, the *A. orana* flight is several weeks later than the *C. pomonella* flight. Thus a reliable control of *A. orana* may not be achieved if the application is timed to the *C. pomonella* flight. Another important aspect to consider in the development of a multispecies product is the possibility of interspecific interaction between the two pheromone systems. In laboratory and field experiments we have demonstrated that the attraction of *C. pomonella* to attracticide sources is disrupted by *A. orana* pheromone released from the same point source (Potting *et al.*, 1999).

CONCLUDING REMARKS

The use of semiochemicals in pest control will undoubtedly become more important. Although semiochemical-based control techniques can not always be applied reliably as a stand-alone control products, they are perfectly suited to be incorporated in integrated pest management programmes or insecticide resistance management programmes. The use of semiochemical-based control techniques could potentially reduce the amount of insecticide by an order of magnitude.

We feel that a sound understanding of the biotic and abiotic factors underlying the semiochemical mediated system we wish to exploit, could greatly improve the success of semiochemical-based pest control strategy. The presented model of attracticide efficacy could constitute a valuable tool in the development of optimal semiochemical-based control techniques.

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Optimizing toxin presentation and acquisition processes

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ABSTRACT

Spatial and temporal presentations of toxins can affect pest encounter and acquisition processes. Deposit structure consisting of deposit size, number and concentration is shown to be of critical importance for both mortality and crop protection. Emerging technologies exacerbate the potential for a wide array of deposit scenarios. Understanding the implications of these technologies on toxin acquisition and pest resistance with the potential interactions with new biologicals, transgenics, and plant health technologies will better prepare agriculture for a more sustainable crop protection future.

INTRODUCTION

Pesticides are applied to agricultural crops for the purpose of protecting plants from damage due to insects, diseases and weeds. The improved efficacy of new actives is remarkable and has allowed doses to be reduced to a few grams per hectare but the capacity to deliver these products efficiently remains suspect. As we take new crop protection chemistries to the field, it is well recognized (and emphasized at previous lab to field symposia), that numerous variables dominate the transfer of toxin from tank to pest. Crop densities, user knowledge, equipment variables, weather (micro and macro), and information on pests (location and density) and target geometry are the obvious factors. With new more complex molecules which are increasingly specific in activity and more vulnerable to environmental parameters, the risks of failure are not diminished. As noted by Hislop (1987), Ford and Salt (1987) and Hall (1997), emerging pesticide application technologies allow more flexibility in delivery parameters and resulting spatial distribution of toxins (Table 1). However, what optima are we able to recommend to the pesticide user, i.e., drop size, and coverage needs beyond the standard "full coverage" label statements? A number of studies have shown that increased coverage did not increase biological results. What is missing? IPM and 'treat as needed' (variable rate technologies and GPS/GIS) are about to enter the agricultural mainstream. What fundamental information requirements on dose, placement, encounter, and dose acquisition are available to enhance the efficiency of the dose-transfer process? There seems to be a vacuum of critical information on how to improve this process which, with new biorationals, seems to require a new approach for identifying and optimizing delivery requirements. Then too, the pest resistance issue has not been resolved.

It is not just drop size and densities that are important, but critical knowledge about pesticides must include information on dose encounter and acquisition, placement precision, and pest behavior in appropriate temporal/spatial settings (Hoy *et al.* 1998). Thus, the quality of the spray deposit and pest behavior encounter processes can illuminate our understanding about current dose-transfer inefficiencies (Hall and Menn, 1999, Hofstein and Chapple, 1999, Bateman, 1999, Evans, 1999, Chapple *et al.*, 1994, and Hoy *et al.*, 1998,

Table 1. Emerging technologies which change toxin distributions on target surfaces.

WHAT	HOW	IMPACT
Drift Reduction	Air-assist, shrouds, low psi, adjuvants, EL and AI nozzles	Deposit structure, large deposits, spatial distribution
Independent control of Volume and Drop Size	Twin fluid, VR nozzles, psi, adjuvants	Deposit concentration, drop size distribution, density, and cover
GPS/GIS	Monitor, site-specific, maps	Macro scale spatial distribution
Pest Detection	Laser, sensors, scouting, etc.	Macro scale spatial distribution
Variable Rate Tech. (VRT)	On-the-go/site specific	Temporal/spatial (scale distributed)
Biotechnology	Plant resistance, value-added genome	Spatial and dose distribution
Decision Models	Trt mgt assistance	Temporal/spatial
IPM	Choice/rate of products	Product changes and dose distribution
Plant Health	Stimulate natural plant defense mechanisms	Spatial/temporal distribution, interactions with conventional plant protection

and Hall and Barry, 1995). This paper summarizes a series of studies which strives to undertake a new approach in revealing the underlying precepts involving lab to field dose, drop size, density issues in order to achieve an improved/predictable field efficacy.

TOXIN PRESENTATION AND ACQUISITION

The development of a model (Pesticide Deposit Simulator – PDS) simulating insect feeding and locomotary behavior of defoliating insect on a leaf surface (Taylor *et al.*, 1993) aided our efforts to document joint effects of drop size, number and AI concentration on efficacy. This strategic model was developed to understand and define potential interactions between deposit structure and insect behavior using diamondback moth (*Plutella xylostella*) feeding on Bt-treated cabbage. Deposit structure is defined as the arrangement of toxin on a treated leaf surface. Atomized sprays deposit toxin on foliage as discrete droplets, which eventually become discrete deposits. The number, size, and the toxin levels within deposits can be used to calculate the total dose applied. Thus, we can assess how insect behavior influences efficacy by interacting with deposit structure to alter dose acquisition. We identified two sets of behavior; (1) chompers which take large bites of foliage from a leaf, and spend little time moving and (2) nibblers which take smaller bites and move frequently (Fig 1). The data were analyzed using a mixture design (modified polynomial regression) as explained in depth by Cornell (1990). Readers are encouraged to review Ebert *et al.*(1999 a, b) with color plates which provide additional clarity to these relatively complex analyses. Deposit size, number and concentration were used to create different mixture levels of deposit structure all with the same total dose applied to a leaf segment. In this study, we were interested in how behavior modified the correlation between % mortality and crop protection. The conclusions suggest the obvious -- % mortality is not always strongly correlated with crop protection. This correlation can be weaker for nibblers, but the variability in response to toxin quality is greater than for chompers. Chompers, which take out large sections of leaf area, seem unable to take advantage of what little deposit heterogeneity remains. This led us to a larger set of experiments (Fig 2).

Figure 1 *Trichoplusia ni* behavior on cabbage: Chompers (left), stay in one place producing large holes; Nibblers (right), move about producing many small holes.

Figure 2. Correlation between crop protection and herbivore mortality shown along with the effect of toxin heterogeneity on herbivore mortality.

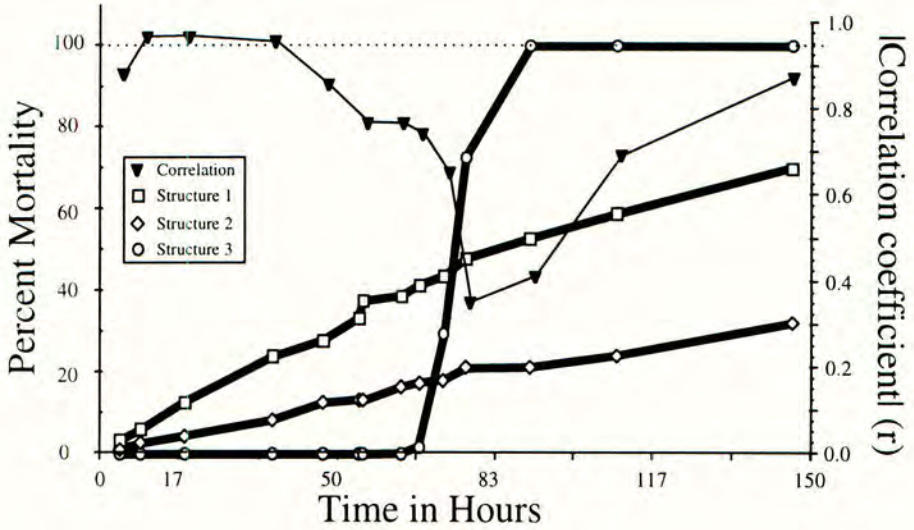

Figure 2 shows the manipulation of the presentation of toxin while maintaining the dose. Structure 1 used a single large deposit with very little toxin in a given portion; Structure 2 used a single very small deposit with toxin concentrated at a single point; and Structure 3 used a large number of deposits with each deposit containing little toxin. Increasing the feeding time increased the effective dose. Figure 2 shows that only 3 has a non-linear response curve, and the relationship between mortality and crop protection is low near the LD_{50} for 3. Traditional bioassays utilize the most uniform structure, exemplified by 3. That mortality is not always highly correlated with crop protection, supports the claim that as we reduce toxin to the minimal efficacious level, we need to be clear on whether our objectives are crop protection or mortality. Ebert *et al.*, (1999 a, b) and Ebert and Hall, (1999) reiterate the hypothesis that dose, drop size and density relationships are critical data gaps if we hope to improve our understanding of how to optimize toxin presentations with these emerging delivery technologies.

TRANSGENIC TOXIN PRESENTATIONS

The recent developments in transgenic crops using Bt protein expression has resulted in an array of questions such as pest resistance concerns; eruption of secondary pests (biodiversity within crops); and speculation about the sustainability of such strategies when optimization of refugia tactics are still being explored. Nevertheless, the development of these technologies which can result in expression of Bt toxins at 10-50+ fold (crop dependent) beyond that of foliar applied Bt suggested that some new pest response studies were needed. To provide initial data on experimentation goals and traits to be examined, we undertook the following studies. In whole plant assays, corn was grown in 4 inch pots; 3 plants per pot and fall armyworm (FAW) (*Spodoptera frugiperda*) neonates were placed in the terminal whorl of each plant. The transgenic corn variety (Novartis Seeds) used in these experiments contains the Bt11 event which expresses the Cry1A(b) protein in all plant tissues. Table 2 shows that the transgenic corn increased pest mortality and reduced feeding rates of FAW as expected.

Table 2. Mortality and weight of Fall Armyworm neonates on normal and transgenic corn.

	Normal	Transgenic
Number of larvae	48	48
Neonate Mortality	18.8%	75.0%
Total Mortality (10 Day)	35.4%	77.1%
Average Larval Weight (mg)	11.497	0.427

Table 3. Mortality and pupal weight of Fall Armyworm fed normal and transgenic corn.

	Pupal Weight (g)	Std Dev	Mortality	Days to Pupation	Std Dev
Normal	0.1560	0.0333	0%	28.00	1.8257
Transgenic	0.1319	0.0141	64%	47.50	6.5574

In a second study, FAW was reared in the laboratory to the neonate stage and placed on agar in petri dishes. Corn leaf sections from Novartis Seeds transgenic corn and the iso line were provided at regular intervals and observations made of insect development through to pupation (Table 3). In addition to the expected mortality as in Table 2, the transgenic corn also reduced pupal weights, and increased the time to pupation (fitness). The variability in pupal weight is greater in normal corn, but the variability in time to pupation is less. This could cause complications in pest control options that rely on timing events for pest control measures.

PLANT HEALTH AND TOXIN RESPONSES

Enhancement of plant defense mechanisms is a recent development which promises to aid pest control strategies by stimulating plants to produce more robust root systems, increase photosynthesis and generally engage a greater tolerance against diseases and pests. For example, US-EPA recently announced (USEPA Office of Pesticide Programs - news release, 1999) the approval of harpin proteins which enhance natural plant defense mechanisms and which are effective against a wide range of plant pests/diseases. This technology poses some interesting research opportunities to understand potential interactions between an increased tolerance and the level of external or internal (transgenic) toxins required to affect pest infestations. Thus, the need for traditional high levels of protectants such as Bt might be diminished where natural plant health is enhanced. LPCAT currently has this under scrutiny using the aforementioned set of experimental tools.

Verkerk and Wright (1996) developed a model on herbivore interactions. Their results suggested a ditrophic interaction with a stomach acting insecticide with high levels of herbivore fitness reducing toxicity and producing a potential synergistic interaction of plant resistance and the insecticide (Figure 3). LPCAT studies confirm these interactions and thus lend credence to opportunities for alternative toxin presentations under enhanced plant health scenarios. Recall, however, that the opportunities for more detailed studies of dose transfer are called for in reviews by Hofstein and Chapple (1999), Evans (1999) on dose transfer of biopesticides, Bateman (1999) and Gerlenter and Evans (1999), who all suggested more specific research/development needs for biopesticides. If we cannot entice the developers to undertake this kind of bold experimentation with conventional pesticides, then it is unlikely that it will happen for the more delicate, biologically specific, biorationals. As noted by P Dowd (pers. com), Bt corn (transgenic) was noted to exhibit 30-40 fold lower levels of fumonisin (mycotoxin) vs non-Bt corn varieties. Mycotoxins like fumonisin, a potential cancer-causing agent, are often found at elevated levels in insect-damaged corn kernels and represent health and export concerns. Consequently, allowing some feeding (pest survival) via a reduced pesticide input strategy may reduce the advantages of clean corn.

In a recent review of toxin presentation, Hoy *et al.* (1998) identify data gaps in the linkage between toxin spatial heterogeneity and insect adaptations to toxins (pest resistance). Data from managed systems show that high doses and more uniform distribution of toxins can select very rapidly for physiological resistance. Alternatively, behavioral avoidance of toxins leads to an effective and stable defense in natural systems. Projecting comparisons from various toxin distributions adapted from Hoy *et al.* (1998), it can be seen how this progression of behaviors at various trophic levels could result in such widely differing results (Table 4). This provides additional evidence of the need for more detailed studies of toxin presentations, especially now that more powerful delivery systems are poised to impact significantly agricultural crop protection systems (transgenics) (Ebert *et al*, 1999 a, b, Ebert and Hall, 1999 and Banken and Stark, 1998).

As illustrated by PDS model scenarios, the issue of pest mobility in a spatial and temporal context adds to the complexity of the toxin presentation and acquisition processes. Toxin spatial distribution, routes of exposure, and pest movement studies thus provide the opportunity and the fundamental conditions for insect behavioral responses and can document the potential for an increased sustainability of crop protection strategies (resistance management).

Figure 3. Conceptual model showing possible ditrophic interactions with a stomach-acting insecticide: a' = high level of herbivore fitness reduces potential toxicity; b' = point of minimum impact on herbivore of combined effects; c' = additive effects of plant resistance; and d' = synergistic interaction on plant resistance and insecticide (after Verkerk and Wright, 1996).

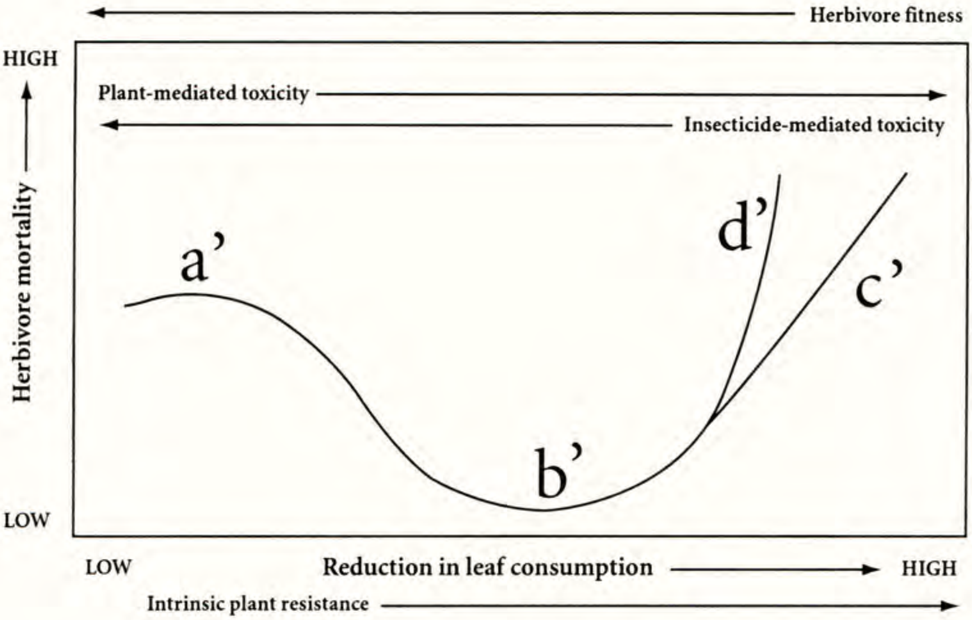

Table 4. Assembly of toxin distribution responses.

TOXIN DISTRIBUTIONS

HETEROGENEOUS	HOMOGENEOUS
Lower concentrations	Higher concentrations
Mobile herbivores Redistributed, reduced damage	Dead or incapacitated herbivores, little damage
3rd Trophic level reinforces movement	3rd Trophic level exploits intoxicated prey or is intoxicated
Behavioral adaptation Further reinforces movement	Physiological adaptation Circumvents plant defense

Spray and count efficacy studies using only mortality observations will not provide the necessary clues to a more sustainable crop protection strategy. However, society/technology developments all stress "convenience to the user" and thus we continue to seek and select easier, simple crop protection strategies rather than more complex decision laden tactics.

The use of GPS/GIS technologies to identify, locate and treat specific sites within fields, for example, has not yet revealed the sustainability of that strategy of treating aggressive population fronts and thus disrupting spatial heterogeneity on the landscape scale. The habitat provided by various possible refugia and subsequent pest resistance response is likely to be site/crop/pest specific. Habitat fragmentation with the addition of buffers/alternate vegetation arenas (for transgenics) changes the mosaic of suitable vs hostile areas for pests. How this landscape of patchiness (toxin vs non-toxin) constrains fitness will require new experimental approaches not heretofore attempted by traditional mortality assessments. Resolving these issues will take measurements of deposit quality on both micro and macro landscape scales and the utilization of such tools as population dynamics, evenness and diversity statistics, and fractal geometry.

Scientists should challenge the "even coverage" goals. Field delivery of toxins is not checked as to the spatial variation actually achieved, which could perhaps alleviate these gaps of understanding of toxin presentation/acquisition.

The "WOW factor" of new technologies is very attractive and serves as human fixation points which lead to the elimination of seeking truths and fundamental understanding of processes. With particulate biopesticides, the assumption that an equal number of particles are in equal sized deposits and hence are equal in toxic transfer potentials is flawed and can add to the coverage dilemma for low volume sprays undertaken by many growers. Field scenarios show large differences in spatial scales as well as goals of the pest control process within habitats.

We suggest that the current plant protection situation requires more detailed information on inputs such as vigor (incoming populations), movement, timing, and the toxin encounter/acquisition process itself compared to the snapshot of a pest population without knowledge of deposit heterogeneity. If we no longer consider the pest control goal as a "biological desert", then the ecological/ecotoxicological details become critical in order to achieve a sustainable practice.

In summary, the presentation of toxins in future agricultural systems is likely to be significantly altered. How placement, deposit quality and pest encounter processes are affected by these changes will impact the longevity of new technologies. The question of "who will undertake this research?" remains unclear as industry continues to merge/downsize and resources in both academia and government diminish and respond to other priorities (crop goals without pesticides). Transgenic technologies however, may add additional pressure on scientists to understand better how to predict more adequately short and long term pest management success under very different toxin placement conditions. We suggest that a deeper understanding of toxin presentation, encounter and dose acquisition processes will greatly aid this predictive capability and is long overdue.

ACKNOWLEDGEMENTS

We acknowledge the contributions made by US EPA via Grant R-823100-01-1, and Novartis Seeds (transgenic corn), and Eden Biosciences (harpin protein) as valuable assets to the studies being undertaken by LPCAT at OSU, Wooster, OH.

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