

Mechanisms and Deployment of Resistance in Trees to Insects

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CHAPTER 9

DEPLOYMENT OF TREE RESISTANCE TO INSECTS IN SHORT-ROTATION *POPULUS* PLANTATIONS

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1. INTRODUCTION

Host plant resistance has been identified as a key component of integrated pest management (IPM) in agriculture and forestry. The topic of deploying and conserving host plant resistance to minimize economic damage caused by insect herbivores is not a new problem. For example, deployment strategies have been addressed in depth in traditional agricultural systems for several decades (see review by Gould 1998). In forestry, however, while the issues are recognized, little rigorous experimental work has been completed. Although theoretical models have been developed to predict the number of clones that are needed to minimize damage and to conserve resistant genes (Libby 1982), actual field tests of these conceptual models are lacking.

To maintain the usefulness of host plant resistance mechanisms as a management tool while at the same time minimizing environmental risk, there are several critical factors to consider: 1) determining and isolating multiple resistance mechanisms to insects, 2) maintaining the effectiveness of host plant resistance in plantation settings over time through adequate lines of resistance and planting designs, and 3) developing resistance to a complex of pests. These factors have even more importance when woody plant species are grown in systems similar to traditional

agricultural crops. At the same time, however, there is the potential to overcome some of the limitations associated with traditional forestry.

The objective of this chapter is to review short-rotation woody crop (SRWC) systems, attempts to incorporate resistance as part of an IPM program, and research and theoretical tests designed to evaluate the effectiveness of host plant resistance deployment strategies. In addition, we compare traditional tree selection and breeding programs for developing tree resistance with genetic engineering approaches. We then provide a detailed review focused on defoliators of short rotation *Populus* plantations, as they have had the greatest insect impact on these systems. However, a review of host plant resistance to other organisms also is discussed, because all potential pests must be considered when developing IPM systems. As SRWC systems are relatively new, but increasing in acreage worldwide, we may see a new complex of organisms emerging that could be even more economically important.

1.1 SHORT-ROTATION WOODY CROP SYSTEMS

SRWC systems are essentially tree plantations that combine traditional agricultural and forestry practices. Most tree plantings usually are done with minimal site preparation following a timber harvest; however, intensive site preparation occurs prior to planting SRWC systems. Site preparation includes tillage to break up the soil, herbicide applications to reduce existing weeds, pre-emergent herbicides to reduce weeds during tree establishment, and the addition of lime or granular fertilizer to increase the pH or nutrient availability of the soil (Dickmann and Stuart 1983). Irrigation or fertigation is often provided, allowing SRWC systems to be grown on less than optimal soils. Typically, trees are planted in a grid pattern; spacing depends on the desired product. Narrow spacing (e.g. 1.5×1.5 m) often is used in coppice plantations, where the end product can be used for pulp or energy (Kenney *et al.* 1993, Peterson *et al.* 1996, Hughes 1997). Wider spacing (e.g. 3×3 m) is used for pulp production and for saw timber where rotation ages are greater (Peterson *et al.* 1996, Zsuffa *et al.* 1996, Eaton 2000b). As the phrase implies, harvest intervals in SRWC systems are much shorter than in traditional forestry and usually range between 1 and 15 years.

Tree species commonly used in SRWC systems are fast growing, early successional species such as *Populus*, *Salix*, *Platanus*, *Liquidambar*, *Eucalyptus*, and *Pinus*. These genera provide species and hybrids that are very shade intolerant; weeds must be kept to a minimum throughout the establishment period by tilling or herbicide applications. Weeds have been shown to have a major negative impact on *Populus* growth in SRWC systems (Hansen *et al.* 1984, Schuette 2000). Most of the species used in SRWC systems are quite susceptible to insect and disease pests. This is common in fast growing tree species, as the majority of the available resources are invested in growth rather than defense (Kozlowski *et al.* 1991). Currently, the most common solution for pest problems in SRWC systems is pesticides. This chapter examines alternative pest management strategies, primarily host plant resistance, that can be used in SRWC systems. Our review is primarily limited to research conducted on *Populus* and *Salix* species in temperate zones;

selections and hybrids from these two genera are the most widely planted trees in temperate SRWC systems.

1.2 SRWC PLANTATION STRATEGIES

Even though no field tests have been conducted on the effect of field size, number of clones needed, or separation distances required between plantings of the same clone (Hall 1993), there is general agreement that there are several plantation strategies that could be used in SRWC systems (Zsuffa *et al.* 1993; Figure 1). Each strategy has positive and negative attributes that might determine which plantation strategy is used in a particular situation. Monoclonal stands, as the name suggests, consist of large, single clone plots. These plots can be up to 20 hectares in size (Hall 1993). Of the four strategies mentioned, monoclonal plantations are the most cost and labor efficient and generally most used by industry (Eaton 2000a). Monoclonal stands are treated much like traditional agricultural crops. The plantations are planted with a single clone, receive the same cultural and chemical treatment, and can be harvested at the same time. However, large monoclonal blocks increase susceptibility to pest problems through resource concentration and ecosystem simplification (*sensu* Root 1973). As a consequence, once a pest becomes established it can continue to proliferate throughout the entire plantation.

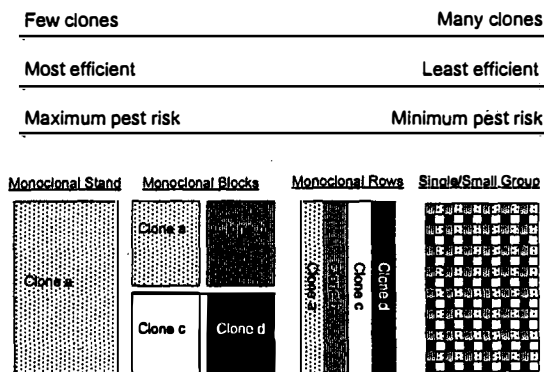


Figure 1. Clonal deployment strategies for short-rotation woody crop systems. (From: Zsuffa *et al.* 1993)

Several additional studies have reviewed the monoclonal block mosaic plantation strategy (Libby 1987, DeBell and Harrington 1993). This consists of several clones, each planted in relatively small monoclonal blocks. These blocks are

planted in a way that no two like clonal blocks are adjacent, creating a checkerboard pattern. This system allows poor performing clones to be continually removed and replaced, if necessary, thus keeping a fully-stocked plantation and constant supply of wood. From a pest management perspective, the monoclonal block mosaic planting pattern is more desirable than pure monoclonal blocks. In the event that one of the clones becomes infested with an insect or pathogen, individual clonal blocks can be managed separately. Diseased or damaged clones can be removed and replanted without disturbing the other clones in the plantation. However, as clone use increases, the logistics of plantation management generally become more difficult.

Clonal rows are another plantation strategy generally used in clonal selection trials and cutting orchards. Clones are planted in several single rows adjacent to one another. This planting method allows the assessment of various growth parameters and pest susceptibility on many clones at one time. Research at Long Ashton, U.K. suggested that mixing rows of susceptible and resistant willow clones may both delay the onset of rust epidemics and reduce the movement and subsequent damage caused by chrysomelid beetles (Royle *et al.* 1998, Peacock *et al.* 1999).

In terms of pest resistance, the safest planting method entails single tree mosaics and small groups of trees. This method is by far the most time and labor intensive to establish (if tree identities are to be maintained), but provides the greatest protection from pests. Single-tree or polyclonal plots also are subject to more inter-plot competition and therefore can result in overall reduced biomass production compared with monoclonal plots (DeBell and Harrington 1997). Libby and Cockerham (1980) also state that single-tree plots can be beneficial for research activities, primarily because they eliminate environmental variances that can occur within plots. However, single-tree plot studies are subject to missing data caused by tree mortality. Also, should a single clone planted in this regime become infested, it is much more difficult to remove the infested plants without harming the other trees.

Based on the above information, it becomes apparent that determining the optimal number of clones necessary to maintain the effectiveness of resistance and prevent plantation failure, while still maintaining plantation efficiency, is a fundamental question that needs to be addressed. (See Robison this volume for an alternative view on clone deployment). Theoretical models suggest that a minimum of seven and perhaps as many as 20 (Libby 1982) or even more than 30 (Roberds and Bisher 1997) unrelated clones are needed. However, using more than 20 clones may create new problems, as it would be difficult to have >20 high-quality clones that do not have some relatedness and therefore a genetic bridge for insect resistance to develop (Libby 1982). Until the early 1990s, clonal diversity had been more apparent than real. For example, although probably several hundred clones have been developed in the U.S., only 3-9 were recommended for nursery production in the Lake States (Hansen *et al.* 1994). Realizing the potential for plantation disaster, most countries and industries have been working to scale up the number of clones available for deployment. In Ontario, the goal was to have more than 50 clones available at any one time with an annual replacement of 5-10% of the clones recommended for planting as problems arose with older clones and/or much more productive clones became available (Hall 1993). Also, clones being deployed are specifically suited to each soil type and planted in monoclonal blocks no larger than

5 ha in size. In Oregon and Washington, U.S.A., only one clone from any full-sib family is taken into production (Hall 1993).

To date, few experiments have attempted to evaluate the effect of multiple-clone deployment strategies on pest populations and damage levels. Nordman (1998) evaluated three clonal deployment strategies on larval gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae). Two *Salix* clones were used: SV1 (*S. dasyclados*, not resistant to *L. dispar*) and SH3 (*S. purpurea*, resistant to *L. dispar*). These clones were arranged in three deployment patterns in the greenhouse: monoclonal blocks, monoclonal mosaics, or clonal rows. Gypsy moth larvae were released in the center of each pattern and allowed to disperse and feed for 10 days. Significant defoliation differences occurred as a result of the clonal deployment pattern. As expected, the least damage occurred on the monoclonal block of the resistant clone SH3. Clonal rows provided better levels of pest resistance than did monoclonal mosaics or the monoclonal block of the non-resistant clone SV1. Presumably, this occurred because larvae spent more time searching for a suitable food source and less time eating. In nature, additional time spent searching for food is detrimental to pests, as their exposure to natural enemies and abiotic hazards is increased.

Peacock *et al.* (1999) demonstrated the spatio-temporal dynamics of a chrysomelid beetle on short-rotation willow in the United Kingdom. Three willow clones (one highly-, one moderately-, and one non-preferred for feeding) were planted as monoclonal blocks or clonal rows. Adult *Phratora vulgatissima* L. (Coleoptera: Chrysomelidae) were shown to aggregate on the preferred clone in both deployment strategies. However, much more time was needed for the beetles to find the preferred clone when in the clonal row pattern, potentially increasing their exposure to natural enemies as well as delaying development.

Another group of strategies related to plantation management includes those borrowed from traditional agricultural systems. These also may work in short-rotation forestry systems. Crop rotation is a common pest management tactic used in agriculture. The same crop or variety is seldom planted in the same field in repeated years, as many pests overwinter in duff or soil within the field or in nearby ground cover or litter. This strategy prevents the pest buildup over several years, as each spring the previous year's food source is not present, causing them to move on or make use of the new crop. This strategy may work in SRWC systems; however, it is not nearly as applicable. For instance, by the end of a *Populus* rotation (8-12 years) new clones are available that are superior to the clones previously planted. Perhaps a more appropriate cultural method would be to leave an area fallow for a year before replanting a SRWC system. *Chrysomela scripta* F. (Coleoptera: Chrysomelidae) are believed to overwinter in leaf litter near their summer food source. By leaving a plantation fallow after harvest, emerging adults would be forced to find a new food source the following spring.

Sage *et al.* (1999) showed that three chrysomelid beetle species overwinter outside *Salix* biomass plantations and re-infested them each spring. Planting a clonal buffer around the desired clones may be a way to control these pests. The buffer rows could be chemically treated or genetically modified with an insect resistant gene while the interior of the plantation could be left untreated, thus creating an insecticidal border around the plantation. This would reduce or

eliminate the beetle population that reached the interior untreated area of the plantation.

Additional clonal deployment strategies with respect to genetically engineered plants will be discussed in section 2.1.4.

1.3 GENETIC ENGINEERING VERSUS TRADITIONAL TREE BREEDING

If the use of host genetics in forest pest management was easily achieved it would probably be more widely used. Research in the use of host genetics to disrupt pest populations has traditionally proceeded through selection and breeding programs. More recently, biotechnology has begun to play an increasingly important role combining the two approaches into a single program (Hart *et al.* 1992). Genetically modified (GM) trees have the potential to substantially increase wood production in the U.S. (Pullman *et al.* 1998). From a pest management perspective, genetically engineering toxic genes into trees seems to be an excellent means of pest control. However, changes created by genetic engineering are very different than those brought on by natural evolution or traditional tree breeding and selection. This tactic introduces organisms into the environment that would not otherwise exist there, providing a potential risk to native flora and fauna. Yet, if properly managed, GM trees have the potential to cause little or no damage to the current state of the environment. Several authors have provided excellent reviews on the benefits and risks of GM trees (Raffa 1989, Boulter *et al.* 1990, McGaughey and Whalon 1992, James 1997, Raffa *et al.* 1997, James *et al.* 1998, Jouanin *et al.* 1998, Pullman *et al.* 1998).

Summarized below are some of the benefits and risks associated with GM trees:

Benefits:

1. Pesticide replacement. The use of GM trees in SRWC systems would reduce the amount of pesticides applied to plantations and subsequently lost into the environment via drift, leaching, etc. (Raffa 1989, James 1997). It has been estimated that only 0.1% of the average pesticide application actually reaches the target pest (Pimental 1995). Trees possessing genetic toxicity to insects would eliminate the pesticide lost (an estimated 99.9%) in the environment while simultaneously providing much more efficient insect control. Furthermore, by not investing the time, effort, and physical resources needed for large-scale pesticide applications, great economic savings could be achieved. GM trees also would provide equal pest protection for the entire growing season regardless of weather (Boulter *et al.* 1990), a luxury rarely attained using chemical sprays, and especially important in managing multivoltine pests.

2. Increased productivity. Trees genetically engineered for pest resistance may not only reduce pesticide cost and input into the environment, but also greatly improve tree productivity by reducing defoliation-related losses. Coyle *et al.* (2002b) showed the negative effect *C. scripta* defoliation had on *Populus* above-ground volume accumulation. Volume was more than 70% greater in chemically

protected trees compared to controls. Also, fast growing trees contribute more carbon sequestration activity than do slower growing trees. The increased productivity potential associated with transgenic trees could help make SRWC *Populus* an effective source of wood and wood-based products.

3. Source of the toxic gene. Most GM *Populus* contain either a *Bacillus thuringiensis* (Bt) gene or a proteinase inhibitor, both of which are naturally-occurring and have relatively well-defined target specificity. Furthermore, these genes are biodegradable and usually non-toxic to mammals, birds, and other vertebrates (Boulter *et al.* 1990). However, because GM trees do not occur naturally in nature, they are not considered true biological control pest management methods even though the pest control tactic they employ occurs naturally.

4. If clonal sublines can be developed with different transgenes for resistance to a particular pest, then plantations could be established as single tree mosaics at the subline resistance level, but as monoclonal blocks from the standpoint of the other commercial traits of a clone (Klopfenstein *et al.* 1993b). This approach would combine the pest management benefits of single-tree mosaics with the logistical and commercial benefits of monoclonal block plantations.

Risks:

1. Biotype evolution. This is defined as the selection for pest populations able to tolerate the new resistance mechanism (Gould 1988). Evolution of new biotypes is the most important risk associated with the deployment of GM plants, and creates an ineffective system in which the GM plant is no longer toxic to the target organism (Raffa 1989, James 1997, Klopfenstein and Hart 1997, Raffa *et al.* 1997). This is particularly problematic when dealing with trees because of their long life cycle. A single SRWC rotation of 8-12 years can encompass enough insect generations to allow new biotypes to develop (James 1997). Also, the transgene is expressed continually in the plant, thus constantly exposing insects to the toxin, a process that accelerates resistance. Bauer (1995) reported on 13 species (including *C. scripta*) that have already developed resistance to Bt in the laboratory.

2. Effects on non-target organisms. The potential for GM plants to adversely affect non-target organisms is of great concern. Perhaps the most well-known and controversial example is the recent finding that corn pollen containing a Bt toxin has the potential to negatively affect monarch butterfly, *Danaus plexippus* L., (Lepidoptera: Danaidae) larval survival and development (Losey *et al.* 1999, Hansen-Jesse and Obrycki 2000). These studies have initiated an intense controversy and increased the number of studies into the non-target effects of transgenic plants. Other studies also have found Bt toxins to negatively affect beneficial organisms (James *et al.* 1993). There also is a risk of predatory insects acquiring toxins through the consumption of contaminated prey. Reducing predator populations this way would not benefit transgenically gained protection, as predator complexes often compliment transgene pest control. Overall, much less research has been conducted on the effects of GM trees on non-target organisms.

3. Transgene escape. Substantial environmental damage could occur if the transgene increases the fitness of the host tree under wild conditions (Raffa 1989, James 1997, Raffa *et al.* 1997, DiFazio *et al.* 1999). The longevity of the escaped GM tree may be greater than that of the original non-GM tree, resulting in increased probability that GM trees could establish in the wild or interbreed with non-GM trees. Hypothetically, GM trees could be more invasive than non-GM trees, thus outcompeting native host trees for available resources. This also could happen if GM trees exhibited an increased growth rate, reaching reproductive maturity earlier than non-GM trees. One way to combat this might be to genetically engineer reproductive sterility. The research group led by Dr. S. H. Strauss at Oregon State University is working on this issue, and has inserted some transgenes that may give sterility (Strauss *et al.* 1995). This could solve many of the risks associated with the deployment of GM trees.

Adequate risk assessment of GM *Populus* is necessary if these plants are to be used in SRWC systems safely and effectively (Raffa *et al.* 1997). Relying on the pest resistance of the transgenic plant alone will almost certainly result in increased biotype formation, thus negating the insecticidal effects of the transgene. Supplemental pest control with pesticide applications, utilizing clonal deployment strategies, and GM crop rotation are effective ways for managing insect resistance.

2. REVIEW OF HOST PLANT RESISTANCE IN *POPULUS* AND SRWC SYSTEMS

2.1 RESISTANCE TO CHRYSOMELID BEETLES

2.1.1 Clonal variation

Developing and selecting insect and disease resistant clones should be emphasized as the primary defensive strategy against pests in short-rotation *Populus*. A great deal of information has already been learned about this group of pests.

Chrysomela scripta is the most important defoliator of *Populus* in the eastern U.S. (Burkot and Benjamin 1979). Both adults and larvae of this multivoltine insect can severely damage branch terminals, and its ability to rapidly increase populations can lead to widespread economic damage in young plantation *Populus* (Harrell *et al.* 1981, Coyle *et al.* 2002b). While synthetic (Abrahamson *et al.* 1977) and biorational (Coyle *et al.* 2000) chemical controls have proven effective in controlling *C. scripta* in plantation *Populus*, these methods are neither environmentally-friendly nor provide a long-term solution to the pest management problem, respectively.

Through the process of selective tree breeding, favorable traits can be combined in superior tree cultivars. This process is ongoing and new clones are continually being developed. Because of the large number of clones potentially available for large-scale clonal deployment, careful screening must be completed to ensure that only clones with positive attributes are chosen. Several general principles have been

established regarding *C. scripta* as a result of clonal screening trials, particularly in the areas of oviposition and feeding preference as well as performance.

Caldbeck *et al.* (1978) examined *C. scripta* feeding preference on 33 *Populus* clones. Both adult and larval *C. scripta* defoliated trees. Visual damage estimates were used to assess beetle preferences. Clonal preferences were observed, and followed lines of sectional parentage. Clones in the section *Populus* (= *Leuce*) (e.g. containing *P. alba* parentage) showed minimal damage, whereas clones in the Aigeiros (e.g. *P. deltoides*) and Tacamahaca (e.g. *P. trichocarpa*) sections had a 22 to 78% reduction in leaf area.

Haugen (1985) evaluated adult *C. scripta* feeding and oviposition preference on 12 *Populus* clones. A multiple choice greenhouse experiment confirmed that adult *C. scripta* did not prefer *Populus* section clones for oviposition. Pure species clones in the sections Aigeiros and Tacamahaca were both highly preferred for oviposition, and there was a relationship between Aigeiros parentage and oviposition preference. Clones with a greater amount of Aigeiros parentage were more preferred for *C. scripta* oviposition than those with greater Tacamahaca parentage.

A study by Bingaman and Hart (1992) showed *C. scripta*'s preference to oviposit on section Aigeiros clones compared to section Tacamahaca clones. Six clones were examined in this study, and two of the top three highest oviposition rates were on clones with section Aigeiros parentage; a section Tacamahaca hybrid was used the least for oviposition. These findings contrast somewhat with those of Haugen (1985), as he found the same section Tacamahaca clone preferred for *C. scripta* oviposition. Furthermore, oviposition was preferred on pure species in sections Aigeiros and Tacamahaca over intersectional hybrids (Bingaman and Hart 1992). Adult *C. scripta* feeding preference also was examined in this study. Increased adult *C. scripta* feeding occurred on clones with a greater percentage of section Aigeiros parentage when compared to clones with section Tacamahaca parentage. However, increased adult feeding was shown on clones in the Aigeiros and Tacamahaca sections when compared in multiple-choice bioassays. A *P. deltoides* × *P. nigra* clone was the most preferred in adult feeding trials; this agreed with Caldbeck *et al.* (1978). Bingaman and Hart (1992) suggested that oviposition preferences followed feeding preferences because adult *C. scripta* spent more time on these clones, thus having a greater opportunity for oviposition.

Previous research showed that *C. scripta* adult feeding preferences did not correspond with larval performance on *Salix* host plants (Orians *et al.* 1997). A study was conducted to determine if adult *C. scripta* preferentially oviposit on clones that increase larval performance. Larval *C. scripta* performance was examined on eight *Populus* clones by measuring larval mortality, pupal weight, adult emergence, and total mortality (Coyle *et al.* 2001). Larvae generally performed better on section Aigeiros × Tacamahaca hybrids as opposed to pure *P. deltoides* or *P. trichocarpa* clones. However, environmental factors may play a more important factor in larval *C. scripta* performance than originally thought, as a seasonal decline in larval performance was evident.

Species in the section *Populus* are not preferred for feeding or oviposition, whether they are pure species or hybrids. Aspens are not natural host plants for *C. scripta* (Baker 1972), and this antixenotic relationship might be exploited by using

selections from the *Populus* section in mixed planting designs. However, section *Populus* clones are quite difficult to cross with clones in other sections, and it is not clear that anything could efficiently come out of a hybridization strategy.

2.1.2 Foliar chemistry

Phytophagous insects usually consider combinations of olfactory, visual, and physical factors when selecting a host plant. Chemical interactions seem to be quite influential in *C. scripta* host plant selection. Trees in the genus *Populus* have a suite of secondary compounds throughout their tissues; most notable are the phenolic glycosides (Palo 1984, Picard *et al.* 1994b, Lindroth and Hwang 1996a). Aspens (*P. tremuloides*) have at least four phenolic glycosides present in their leaf tissues (salicin, salicortin, tremuloidin, and tremulacin), whereas *P. deltoides* seems to lack tremulacin (Lindroth *et al.* 1987). Extensive research on aspen phenolic glycosides has come from Dr. R. L. Lindroth and his research group at the University of Wisconsin. Lindroth and Hwang (1996b) have written a comprehensive review of this subject.

Seasonal fluctuations in the amounts of phenolic glycosides and nitrogen content occur in several *Populus* species and clones (Dickson and Larson 1976, Lindroth *et al.* 1987, Osier *et al.* 2000b). Generally, these chemicals decline over the course of a growing season. However, younger leaves had significantly higher concentrations of phenolic glycosides than did older leaves (Lindroth *et al.* 1987). Seasonal decline also was found in leaf surface long-chain alcohol and α -tocopherylquinone (α -TQ) concentrations (Coyle 2000).

Herbivory by chrysomelid beetles also influenced *Populus* foliar chemistry. *Populus tremula* $\times$ *P. tremuloides* clones showed an increase in phenolic glycoside production in response to *C. scripta* defoliation (Picard *et al.* 1994a). This resulted in decreased herbivory by the beetle. Bingaman and Hart (1993) examined this relationship with *C. scripta* and hybrid *Populus*. Phenolic glycoside (salicin, salicortin, and tremulacin) content was measured on seven hybrid *Populus* clones. Chemical amounts varied among clone and leaf age class (younger leaves had higher phenolic glycoside concentrations). Tremulacin amounts in hybrid *Populus* were negatively correlated with *C. scripta* feeding and oviposition preferences, whereas salicin and salicortin amounts were not.

Matsuda and Matsuo (1985) showed that some phenolic glycosides in *Salix gracilistyla* leaves act as chrysomelid beetle feeding stimulants. Long-chain alcohols on the leaf surface also can serve as phagostimulants to chrysomelid beetles (Adati and Matsuda 1993). Recently, Lin *et al.* (1998a) discovered a suite of *Populus* leaf surface chemicals that act as adult *C. scripta* phagostimulants. Long-chain alcohols (C_{22} - C_{30}) and α -TQ were isolated from the *P. deltoides* $\times$ *P. nigra* clone 'Eugenei'. Artificial leaf disc bioassays were used to examine the effects of these chemicals on adult *C. scripta* feeding. Alcohols or α -TQ alone did not stimulate intense feeding behavior, but when used in specific ratios these chemicals induced *C. scripta* to bite. A subsequent study (Lin *et al.* 1998b) investigated the interactions between *Populus* leaf surface phagostimulants from field-grown trees and adult *C. scripta*. Adult *C. scripta* feeding preferences were examined for 91

Populus clones from a University of Washington pedigree line. Feeding preferences were then correlated to the aforementioned phagostimulants and to the phenolic glycosides tremulacin and salicortin. Leaf surface chemical amounts of long-chain alcohols and the phenolic glycosides did not explain *C. scripta* feeding preferences. However, feeding preference was linked to α -TQ content. Leaf area consumption increased as α -TQ concentrations reached 2.5 ng/cm², but feeding decreased when α -TQ levels increased further. This study linked the α -TQ content on the *Populus* leaf surface to adult *C. scripta* feeding preference.

It is unknown if *Populus* trees are able to alter their leaf surface phagostimulant concentrations in response to *C. scripta* feeding. A study was conducted to examine the effects of larval *C. scripta* defoliation on the leaf surface phagostimulant concentrations of eight *Populus* clones. Coyle (2000) found that amounts of these chemicals were not significantly altered by larval *C. scripta* defoliation. Larval performance did not correlate with leaf surface phagostimulant concentrations; however, both larval performance and leaf surface chemical amounts declined over the course of a growing season.

There are many implications and applications from this research. Adult *C. scripta* *Populus* clonal preference is mediated by α -TQ amounts on the leaf surface; however, it is unknown if *Populus* clones preferred by adult *C. scripta* for feeding and oviposition are more suitable for larval growth and development. If this proves true, clones less preferred by adult *C. scripta* could be used in *Populus* breeding and clonal selection programs. This could, theoretically, produce clones less suitable for larval *C. scripta* growth and development. Also, the identification of *C. scripta* phagostimulants can be used in many ways. Efforts have been made to develop an artificial diet for *C. scripta* (Bauer 1990) as maintaining a laboratory colony is labor intensive and requires much greenhouse space for food trees. The diet developed by Bauer (1990) was nutritionally adequate, but resulted in decreased colony health. Attempts to incorporate *Populus* leaf surface phagostimulants into the diet to increase *C. scripta* consumption have been unsuccessful (Coyle and Hart, unpublished data).

2.1.3 Proteinase inhibitor genes

Genetic engineering of woody plants is a relatively new technology. Currently, the primary focus is on incorporating genes for pest resistance (Heuchelin *et al.* 1997, Klopfenstein and Hart 1997). *Populus* trees are easily manipulated through various genetic engineering methods (Klopfenstein *et al.* 1997b). Effective procedures have been developed for vegetative propagation of *Populus* (Faltsonson *et al.* 1983). The ability to genetically manipulate a rapidly growing woody plant with the possibility of quickly increasing plant numbers opens doors to new pest management strategies. Scientists at Iowa State University successfully transformed a hybrid poplar clone, *P. alba* $\times$ *P. grandidentata*, with a wound-inducible proteinase inhibitor II (*pin2*) gene from a potato (Klopfenstein *et al.* 1991). Field data showed that transgenic plants did not statistically differ in height or diameter compared to non-transformed controls (Klopfenstein *et al.* 1993a). This study showed that pest resistance genes can be incorporated into *Populus* clones without compromising growth.

Several studies have shown successful incorporation and application of the *pin2* gene in hybrid poplar. Reduced leaf area consumption and growth rates occurred when larval *Plagiodera versicolora* Laicharting (Coleoptera: Chrysomelidae) were fed *Populus* leaves from clones transformed with the *pin2* gene (Klopfenstein *et al.* 1994, 1997a). Kang *et al.* (1997) examined transgenic *Populus* resistance to *C. scripta* on tissue-culture plantlets of the Belgian clone Ogy (*P. deltoides* × *P. nigra*) and three sublines transformed with the *pin2* gene. Leaf area consumption was reduced by as much as 45% and larval *C. scripta* weight was up to 16% less on the transformed lines compared with Ogy.

Chrysomela scripta resistance on these clones was evaluated in a greenhouse experiment (Coyle *et al.* 2002a) to determine if the resistance exhibited in plantlets was maintained in young trees. Leaf area consumption and larval *C. scripta* growth and development were not affected by the presence of the *pin2* gene. We can provide several explanations for these results, with environmental differences posing as the main concern when comparing these two studies. Kang *et al.* (1997) placed larvae in test tubes with the plantlets, whereas Coyle *et al.* (2002a) conducted their study in the greenhouse. There are many differences between these two environments, including sterility, air composition, and their effects on plant tissue growth. Large increases in plant size accompany the laboratory – greenhouse – field study experimental sequence; this may have had an effect on the efficacy of the *pin2* gene. It is not known if a field study would produce results similar to either of the previous studies.

European scientists also have had success incorporating proteinase inhibitor genes into *Populus* for chrysomelid resistance. Leple *et al.* (1995) showed high levels of gene expression in *P. tremulae* × *P. tremuloides* clones transformed with a proteinase inhibitor. Transformed clones were toxic to *Chrysomela tremulae* F. larvae, an important pest of poplars in Europe. This was the first study to successfully transform *Populus* with a proteinase inhibitor and demonstrate toxicity to chrysomelid larvae. Recently, a proteinase inhibitor gene was incorporated into *P. alba* (Delledonne *et al.* 2001) and high levels of resistance to *Chrysomela populi* L. were obtained.

While this technology seems promising, there are still several cases of plants transformed with proteinase inhibitor genes not showing resistance to insect herbivores (Confalonieri *et al.* 1998, Girard *et al.* 1998, Coyle *et al.* 2002a). Insects do possess the ability to adapt to proteinase inhibitor genes just as any other transgenic control method (Jongsma and Bolter 1997).

2.1.4 Transgenic Bt trees

Technology has advanced to the point that select Bt toxin genes can be inserted directly into plants for insect control. This pest management tactic is not new to traditional agricultural crops; Bt corn has been commercially sold and field planted since 1995 (Carozzi and Koziel 1997). Currently, cultivars of soybean, cotton, potato, and a host of other crop species have been developed and are registered for use.

The use of transgenic Bt-expressing plants in forestry systems is still a novel approach to pest management. While the application of Bt formulations to plantation *Populus* is an effective way to combat pests (Coyle *et al.* 2000), the use of transgenic trees can be more favorable for a number of reasons (Meilan *et al.* 2000). Spray drift is reduced, as the Bt toxin is produced in the plant tissues, and the toxin will not degrade as will a sprayed formulation. Also, Bt plants only expose the toxic gene to insects feeding on the plant. Bt toxins differ from proteinase inhibitors in that Bt generally causes direct mortality of the target pest. Proteinase inhibitors have a more subtle mode of action, reducing fecundity, extending the life cycle, and causing reduced weight and changes in insect behavior (Ryan 1981, 1990).

Populus was the first woody plant species to be transformed with the Bt gene (McCown *et al.* 1991). Researchers at Oregon State University have since produced over 1,700 transgenic *Populus* lines (Strauss *et al.* 1998). Resistance to *C. scripta* was evaluated on 51 lines of Bt *Populus* transformed with an *Agrobacterium tumefaciens* vector (Meilan *et al.* 2000). Every transformed strain evaluated showed significantly reduced defoliation and increased growth at the end of one growing season. Robison *et al.* (1994) and Wang *et al.* (1996) observed insecticidal effects in poplars with a lepidopteran-specific Bt gene. Large-scale field trials are currently being initiated by Meilan *et al.* (2000) in the Pacific Northwest and by Wang *et al.* (1996) in China.

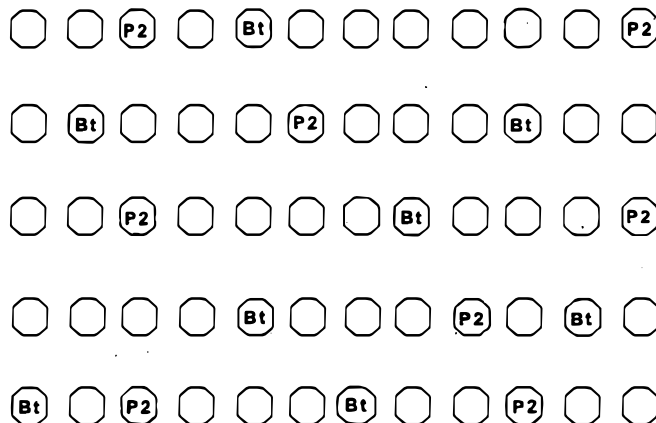


Figure 2. The high dose/refuge strategy (modified from Klopfenstein *et al.* 1993b). Trees with Bt contain a Bt gene, and trees with P2 contain a proteinase inhibitor. The toxins are at a concentration much greater than needed to achieve 100% mortality. Mixing toxins (as in this figure) provides an added measure of protection against insecticide resistance.

Reviews by Tabashnik (1994) and Gould (1998) provide excellent summaries of insect resistance management with respect to transgenic crops. These reviews emphasise several resistance management tactics, including mixtures of toxins, refugia strategy, high dose strategy, synergists, and spatial and temporal expression of toxins. The refuge strategy may be the best way to slow the development of insect resistance in transgenic crops (Tabashnik 1994). This strategy employs a refuge of non-transgenic plants planted with transgenic ones. Non-resistant pests are allowed to reproduce without receiving sublethal doses of the toxin, and subsequently breed with resistant pests. This diffuses the gene for insect resistance, thus delaying the species' or population's development of resistance. Gould (1998) focuses on the high dose/refuge approach for management of insect resistance to Bt crops (Figure 2). This differs only in that the transgenic plants present with the refuge carry a level of toxicity much greater than needed to kill the pest. If the level is sufficiently high enough, even resistant individuals will be killed by the toxin, and will not be able to pass the resistant gene to future generations. While refuges can be within or adjacent to transgenic plots, adjacent refuges have been shown to be more effective in some systems (Tang *et al.* 2001).

2.2 RESISTANCE TO OTHER DAMAGING AGENTS

There is considerable interclonal variation in secondary chemical amounts present in *Populus* foliage (Lindroth and Hwang 1996a, 1996b, Hwang and Lindroth 1997, Lin *et al.* 1998b, Osier *et al.* 2000b). Insect survival, larval stadium duration, and body weight varied greatly for two larval lepidopterans, *L. dispar* and *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae), when reared on 13 different aspen clones (Hwang and Lindroth 1997). These differences were more pronounced in *L. dispar*, where larval survival ranged from 0 to 100% in fourth instar larvae. Fourth instar *M. disstria* survival ranged from 60 to 100% on the same clones. *Lymantria dispar* performance was reduced when higher phenolic glycoside concentrations were present in *P. tremuloides* foliage (Osier *et al.* 2000a). However, foliar consumption was positively correlated with tannin concentration.

Atmospheric CO₂ and light intensity also may influence larval performance and foliar chemistry. Increased CO₂ and light levels induced increases in aspen foliar phenolic glycoside content (Lindroth and Kinney 1998, Roth *et al.* 1998, McDonald *et al.* 1999). Furthermore, *L. dispar* larvae had reduced growth rates when fed aspen leaves exposed to increased CO₂ levels (Lindroth and Kinney 1998, McDonald *et al.* 1999). Overall, these larval performance reductions were not great, and were not apparent in one study using *M. disstria* (Roth *et al.* 1998).

Many studies have shown that insect defoliation can induce chemical changes in foliage. Often these are specific damage-induced chemical changes that provide the plant protection (Lindroth and Hwang 1996b). Aspen trees have a well-documented wound-induced chemical protection system. For instance, defoliation by *Choristoneura conflictana* (Walker) (Lepidoptera: Tortricidae) induces increases in phenolic glycoside content (Clausen *et al.* 1989). These chemicals are toxic to *C. conflictana*, and result in reduced larval performance. Larval *L. dispar* and *M. disstria* defoliation induced increases in aspen phenolic glycoside content (Lindroth

and Kinney 1998, Roth *et al.* 1998). However, only *L. dispar* performance was correlated negatively with phenolic glycoside content in aspen. Therefore, wound-induced increases in foliar phenolic glycosides are an effective but species-specific natural defense mechanism.

Several additional insect pests, including root and shoot borers, sawflies, lepidopterans, and leaf miners, attack *Populus* plantations (Solomon and Abrahamson 1972, Thomas and Rose 1979, Wilson 1979, Solomon 1988, Sage and Tucker 1997). However, their populations generally do not reach the economically damaging levels of *C. scripta*, *M. disstria*, or *L. dispar*. However, the cottonwood twig borer, *Gypsonoma hainbachiana* (Kearfott) (Lepidoptera: Tortricidae), can have an economic impact on SRWC systems. Morris (1967) identified this insect as a potentially damaging pest of plantation *Populus*. High populations have been shown to cause nearly 100% terminal shoot mortality (Stewart and Payne 1975). Current controls include synthetic chemicals (Morris 1960, Coster *et al.* 1972) and possibly natural biological controls (Morris 1967). Two studies have evaluated *P. deltoides* clonal resistance to *G. hainbachiana* (Woessner and Payne 1971, Payne *et al.* 1972). Pure *P. deltoides* clones in both studies were more heavily attacked than a hybrid clone. These results imply that *Populus* hybridization may be required in order to attain resistance to *G. hainbachiana*, a phenomenon observed in other herbivory studies and reviewed by Fritz *et al.* (1999). However, McMillin *et al.* (unpublished data) found varying damage levels among four hybrid *Populus* clones and across three locations in Iowa (Figure 3), indicating that not all hybrid poplars are resistant to *G. hainbachiana* damage.

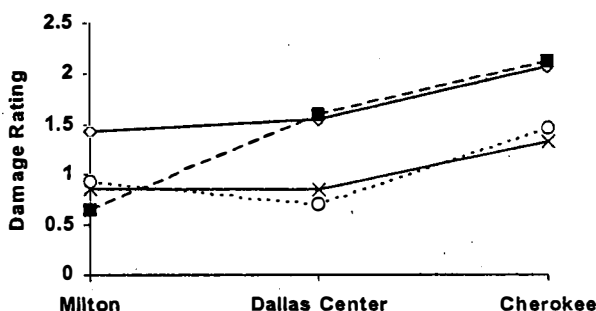


Figure 3. Damage caused by *Gypsonoma hainbachiana* to *Populus* clones at three sites in Iowa, USA. *P. deltoides* x *P. maximowiczii* (open diamonds), *P. nigra* x *P. maximowiczii* (solid squares), *P. deltoides* x *P. nigra* (crosses), and *P. deltoides* x *P. deltoides* (open circles). Damage ratings based on a 0 – 5 scale of increasing damage to terminal leaders.

Recently, McMillin *et al.* (1998) suggested that increasing the number of actively-growing *Populus* terminals in a plantation can result in *G. hiambachiana* population increases. Hence, alternate controls must be attained for this pest, as plantation size will most likely increase as short-rotation forestry gains acceptance and industrial implementation.

Disease also is a major issue in short-rotation *Populus* plantations, and the resulting damage can be equal to or greater than that caused by insects, particularly in the establishment years (Ostry and McNabb 1985). Disease can be managed much the same as insect pests by incorporating host-plant resistance mechanisms and utilizing proper plantation planting and management strategies. Many diseases can damage *Populus* (Berbee 1964, Shea 1971, Ostry and McNabb 1985, Newcombe 1996). However, only a few are of great economic significance.

Melampsora medusae Thuern. (Uredinales: Melampsoraceae) leaf rust is one of the most damaging and widely studied diseases of *Populus* in North America (Newcombe 1996). This disease can result in premature leaf abscission, reduced growth, increased lateral branching, and early mortality (Newcombe and Chastagner 1993, Newcombe *et al.* 1996, Callan 1998). Previous studies have shown wide variation in *Populus* resistance to *Melampsora* rust (Ostry and McNabb 1985, Newcombe *et al.* 1994). However, it is becoming clear that selection for complete resistance and the deployment of resistant clones has led to the development of many new rust biotypes that may overcome host resistance.

Septoria musiva Peck (Dothideales: Sphaerioidaceae) leaf spot and stem canker is an important cosmopolitan disease of *Populus*. This fungus generally occurs as leaf spot on native *Populus* with cankers predominately reported on clones containing Tacamahaca parentage (Bier 1939, Waterman 1946). Stem breakage often occurs well before harvest age, and susceptible clones are generally not utilized in plantations (Ostry *et al.* 1989). Selecting *Populus* clones resistant to *Septoria* through clonal screening is the only way to combat this pest. Clones with Tacamahaca parentage have been shown to be more susceptible than clones with Aigeiros parentage (Ostry and Berguson 1993). This study also showed that selecting canker resistant clones can result in reduced biomass accumulation. However, recent clone selection work with pure *P. deltoides* germplasm indicates that high yields can be combined with *Septoria* canker resistance (Hall, unpublished data).

Increased growth is often sought by hybridizing *Populus* rather than planting a single species (Stettler *et al.* 1996). However, hybridization may sacrifice any natural disease resistance occurring in native species (Fritz *et al.* 1999). Presently, the only effective way to combat pathogens is by utilizing host plant resistance mechanisms in breeding and selecting resistant clones (Callan 1998). Genes conferring resistance to *Melampsora* have been discovered in hybrid and pure species *Populus* (Newcombe *et al.* 1996, Tabor *et al.* 2000). Selective breeding and/or genetic engineering may facilitate the incorporation of these genes into new *Populus* clones. Exapted resistance (that conferred by a non-native species) may play a much larger role in future *Populus* plantation management (Newcombe 1998). This type of resistance will need to be used in concurrence with other

resistance mechanisms to effectively combat many of the pathogens occurring on *Populus*.

2.3 MULTIPLE PEST SCREENING

All woody crop species have a varied complex of organisms that have the ability to limit the economic viability of these systems. For example, *Populus* has at least 24 insect defoliators as well as several pathogens in North America (Dickmann and Stuart 1983). Further complicating the development of deployment strategies for *Populus* resistance is the fact that different insect species seem to prefer different clones; a resistant clone to one insect is susceptible to a complex of other insects. Thus, screening of advanced clones or selections must include tests across a variety of taxa.

Several studies have screened clones for resistance and/or susceptibility to two or more defoliating insects and pathogens. Researchers at the University of Wisconsin screened three *Populus* clones to *C. scripta* and *M. disstria* (Raffa *et al.* 1991, Ramachandran 1993). Two clones were highly susceptible to *C. scripta*, but resistant to *M. disstria*, whereas the opposite was true in the third clone. This trade-off was caused by the high phenolic glycoside content in *M. disstria* resistant clones, which positively influences *C. scripta*, and the low concentration in the *M. disstria* susceptible clone. This research suggests that the *M. disstria* resistant clone could be engineered with the coleopteran-toxic Bt gene to create resistance across two taxa (Raffa *et al.* 1991, Ramachandran 1993).

Nordman (1998) screened 19 *Salix* and six *Populus* clones for resistance to seven defoliating insects. Two primary results were obtained: a wide range of susceptibility occurred among clones to a particular defoliator, as well as in individual clones to all seven defoliators. Clones resistant to one species often were not resistant to others. This study demonstrates the need for multiple pest resistance screening when preparing clones for commercial use.

3. DEVELOPMENT OF AN IPM PLAN FOR *C. SCRIPTA*

The majority of SRWC commercial hardwood operations have been developed in the Pacific Northwest region of the U.S. and in Europe, but more recently are being established throughout several other locations in the U.S. and Canada (van Oosten 2000). Present management methods for insect pests, such as *C. scripta*, in commercial plantations are currently quite dependent upon applications of broad-spectrum organic or biorational insecticides. Often, this is still done on a calendar schedule, but commercial growers are beginning to recognize the desirability of monitoring for pest activity and levels and timing applications to increase efficacy and reduce losses. They are becoming aware that repeated applications of a single pesticide may contribute to the development of insect resistance to that material. The development and initiation of an IPM program is the next step in *Populus* pest management. IPM is designed to be more environmentally-friendly and incorporate many different control measures into a pest management strategy. IPM also may help reduce the development of insect biotypes and thus prolong the effective

commercial life of desirable clones. With some expansion and fine tuning of the existing base of knowledge, we seem to be poised to develop and implement an IPM plan for plantation *Populus*, particularly for managing *C. scripta*.

Plantations should contain resistant clones, as this will serve as the foundation for *C. scripta* control. By selecting clones that are less preferred for adult feeding and oviposition we can reduce the amount of time adult beetles spend on the clones, therefore, reducing their tendency to oviposit (Bingaman and Hart 1992). Also, clones that cause poor larval performance and delayed development can be planted to reduce the number of adults present. As stated earlier, multiple lines of resistance are needed to prevent biotype evolution. However, the number of lines needed is still under debate. Unless clones can be developed that are completely resistant to *C. scripta*, other pest management strategies will also need to be utilized.

Biorational sprays are a chemical control method capable of providing excellent *C. scripta* control. Unlike their synthetic chemical counterparts, biorational sprays containing Bt are environmentally-friendly and non-toxic to vertebrates and most non-target organisms (Tabashnik 1994). Several studies have shown Bt toxins to be extremely effective in controlling *C. scripta* populations in the laboratory (Bauer 1990, James *et al.* 1999). Coyle *et al.* (2000) achieved excellent *C. scripta* control in the laboratory using two commercially available Bt formulations. These formulations were then applied to a *Populus* plantation and similar results were attained. Results from Coyle *et al.* (2000) agreed with previous laboratory studies (Bauer 1990, James *et al.* 1999) in that susceptibility to Bt was negatively correlated with beetle age and size. Adult *C. scripta* are far less susceptible to Bt than are larvae, especially the first two instars. Hence, the timing of Bt application is of great importance. Bt formulations will achieve the greatest level of control early in the *C. scripta* generation, and should be applied when eggs and first instars are the predominant life stages. Coyle *et al.* (2000) made their applications when there were equal numbers of unhatched and freshly hatched egg masses. After three seasons, *Populus* trees protected by Bt sprays had produced between 50 and 73% more above-ground volume than their unprotected counterparts (Coyle *et al.* 2002b).

Because the early life stages are the most vulnerable, population monitoring is an essential aspect of *C. scripta* management. Coyle *et al.* (2000) visually determined *C. scripta* life stages; however, this was time consuming and required experienced personnel able to recognize the various life stages. Nebeker *et al.* (2001) used boll weevil traps to monitor adult *C. scripta* field populations. Traps provided information on population levels and the current life stage in the field. This information could be used in conjunction with biorational sprays. *C. scripta* emergence was easily detectable, as extremely large numbers were caught within 1-2 days. After emergence, adult *C. scripta* undergo a 5-7 day feeding period during which they become sexually mature (Burkot and Benjamin 1979). Traps can indicate when each beetle generation emerges, and plantation managers can use this information, along with the knowledge of the maturation feeding period, to best predict the most optimal time to apply treatment.

Degree-day (DD) calculations also can be used to predict appropriate spraying times. Burkot and Benjamin (1979) found that *C. scripta* generations required between 222 and 273 DD depending on the generation and temperature. Similarly,

Jarrard (1997) examined the DD requirements on *C. scripta* in Iowa. This study observed requirements of between 229 and 317 DD per generation. Jarrard (1997) also found that observed DD requirements were very similar (all within 35 DD) to model-based predicted values. Two studies examined *C. scripta* DD requirements in Mississippi, USA. Pope and Nebeker (2002) and Nebeker *et al.* (2001) required 281 DD and 280 DD, respectively, for one *C. scripta* generation. Their results agreed closely with those of Jarrard (1997). This information is of significant importance. Because DD models seem to be adequate predictors of *C. scripta* life stages, we can estimate when each *C. scripta* generation will occur in the field and treat accordingly. Jarrard (1997) found that predicted DD requirements were within two calendar days of development observed in the field. This information can be used to create a better spray schedule based on insect life stage rather than on a strict calendar schedule. This will save not only time and money, but will reduce insecticide applications to plantation *Populus*.

Insecticide applications can be reduced further by incorporating an accurate economic injury level (EIL) (Pedigo *et al.* 1986) for *C. scripta* on plantation *Populus*. Fang and Hart (2000) examined the relationship between larval *C. scripta* population levels and subsequent plant damage. This study also showed that egg mass densities may be a useful indicator of potential defoliation. A concurrent study by Fang (1997) integrated several factors and derived an EIL for *C. scripta* of 0.2 – 0.9 egg masses per terminal for the second generation. Populations below this level were not predicted to cause sufficient economic loss to justify pesticide application. Economic gain would occur only when populations or damage above the EIL were treated.

Natural enemies do contribute to *C. scripta* population control, but seemingly not to a great extent in commercial plantations. Burkot and Benjamin (1979) examined the natural enemies responsible for *C. scripta* mortality in Wisconsin. Natural enemies had the least effect on *C. scripta* populations during the first *C. scripta* generation, yet exhibited greater control in successive generations. This was primarily accomplished by coccinellid predation on egg masses and pupal parasitism by a parasitic wasp, *Schizonotus latus* Walker (Hymenoptera: Pteromalidae). *Schizonotus latus* parasitized over 25% of the pupae during the third *C. scripta* generation, and coccinellids accounted for up to 25% of egg mortality in the fourth generation. Jarrard (1997) examined the natural enemy complex in central Iowa. Similar to the study in Wisconsin, coccinellid predators were the most numerous *C. scripta* natural enemy. The greatest natural enemy influence occurred on egg masses in the second *C. scripta* generation, where over 70% mortality was recorded. Thus, natural enemies do exert control on *C. scripta* populations, primarily in the sessile (egg and pupal) stages. However, because of the multivoltine lifestyle and reproductive potential of *C. scripta* (Coyle *et al.* 1999) natural enemies alone do not seem to be able to control populations effectively in plantations.

In summary, many components of an IPM program for *C. scripta* have been developed. What is needed is the integration of all the pieces of the puzzle together in different planting strategies for at least one rotation. This could serve not only to test the accuracy of the information elucidated to date, but would serve as a benchmark to determine the most effective directions for additional research.

4. CLONAL DEVELOPMENT AND DEPLOYMENT STRATEGIES FOR TODAY AND FUTURE RESEARCH NEEDS

Based on our experience to date, the best clonal development strategy appears to be to breed and select for tolerance and/or resistance to *Septoria* and *Melampsora* diseases and then genetically transform the best clones with the Bt genes or proteinase inhibitors and other insect resistance mechanisms.

The mosaics of monoclonal stands deployment strategy seems to have the most potential for large-scale use at this time (Zsuffa *et al.* 1993), while the high dose/refugia strategy should be added as soon as the problems with commercial plantings of transgenic clones are satisfactorily addressed. Matching clones to specific soil conditions or production objectives will increase the efficacy of these systems further. An obvious, but sometimes overlooked, issue is that clonal deployment strategies can not overcome poor quality clones if they are used in plantings (Zsuffa *et al.* 1993).

To ensure clonal diversity and therefore protect clone longevity and plantation success, Hall (1993) recommends the following: 1) establish a maximum block size that can be planted to one clone, 2) prohibit two fields of the same clone from bordering one another, and 3) within any one growing region, no clone should make up any more than 15% of the plantings in any given year. Empirical research on commercial plantations will probably be the only practical way to refine these recommendations.

While we believe that host plant resistance is an important pest management tool, other aspects of IPM also should be examined in order to effectively utilize the various approaches available and reduce the selective pressures on the target pest (Hart *et al.* 1992). Incorporating effective clonal deployment strategies with host plant resistance and other IPM tactics will reduce selective pressure even further. We also must remember that SRWC plantations are delicate biological systems. Systematic monitoring for the efficacy of the resistance trait is necessary, as is the continued search for potential new pests and new pest management tactics.

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