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# Integration of management tactics for the rice water weevil *Lissorhoptrus oryzophilus* Kuschel with particular emphasis on use of plant resistance (tolerance)

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**INTEGRATION OF MANAGEMENT TACTICS FOR THE RICE WATER  
WEEVIL *LISSORHOPTRUS ORYZOPHILUS* KUSCHEL WITH PARTICULAR  
EMPHASIS ON USE OF PLANT RESISTANCE (TOLERANCE)**

A Dissertation

Submitted to the Graduate Faculty of the  
Louisiana State University and  
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Doctor of Philosophy

in

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by

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## ABSTRACT

Studies were conducted to test the hypothesis, significant benefits can be achieved for the management of rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, by the use of rice varieties with low levels of host plant resistance coupled with the judicious use of cultural practices and insecticides. Tolerant traits of a current commercial variety of rice, "Bengal", was manifested consistently over multiple years. Study on rice growth as effected by weevil injury showed that pruning of root systems by larvae of weevil resulted in a decrease in tiller number and shoot biomass of rice plants in the vegetative stage of growth. Yield losses were due to a combination of decreases in panicle densities, numbers of grains per panicle, and grain weights. Experiments on density-yield relationships documented that larval densities during earlier stages of rice growth were more strongly correlated with yield losses than were larval densities later in the growing season. Slopes of yield loss were greater negative for early-flood than for delayed-flood plots, and greater negative for susceptible varieties. A phenology model predicted that emergence of weevils occurred after the accumulation of 139.2 degree-days ( $^{\circ}\text{C} * \text{Day}$ ). Using a temperature threshold of  $10^{\circ}\text{C}$ , the total degree-days required for development of one generation is about 623.4 degree-days (from egg to pupation was  $359.1 \pm 19.4$ ; pupal development was 264.3). Larval development did not differ between varieties with different resistant levels. In conclusion, this study showed that host-plant resistance, delayed-flooding and reduced insecticide can be integrated together. Although use of high rate of insecticide currently gives the best economic return, integrated control provides a combination of adequate control and environmental

friendliness. The same economic return can be achieved using a more tolerant variety and delayed-flood without insecticide treatment.

# CHAPTER 1

## INTRODUCTION

### IPM-The Big Picture

There are many definitions of Integrated Pest Management (IPM). A recent definition states "IPM is a decision support system for the selection and use of pest control tactics, singly or harmoniously coordinated into a management strategy, based on cost/benefit analyses that take into account the interests of and impacts on producers, society, and the environment." (Kogan 1998). An IPM program seeks to design cost-effective management strategies that rely on a consideration of the whole context of a farming system and a balanced use of control tactics. The tools of IPM include chemical tools (pesticides, insect growth regulators and pheromones), biological tools (conservation, enhancement, or release of natural enemies), cultural practices (crop rotation, cultivation, irrigation, pest monitoring, etc.) and genetic tactics (sterile release, resistant varieties, transgenic plants). The goal of IPM is to ensure the production of high quality natural products in an economic manner and to minimize risks to human health and environment.

A fundamental principle of IPM is that natural processes can be manipulated to maximize regulation of pest populations in an agroecosystem, and that chemical controls should be used only when and where natural processes of control fail to keep pests below economic injury levels (National Research Council 1996). An agroecosystem is dynamic with interactions of physical, chemical and biological processes. The coexisting crops, herbivores, predators, pathogens, weeds, and other organisms interact with one another and respond to their environment. In a natural system, this web of interactions contributes to its stability. In natural systems, such as a mature forest, the population of a

species is elegantly regulated by multiple interacting forces such as natural enemies and limited food resources. Such stability (low variance in density of pests over time) is an essential feature of a successful pest management program (National Research Council 1996). However, the ecological principles that apply to a mature natural system may not always apply to agricultural ecosystems because agricultural ecosystems display rather loose trophic interconnections (Horn 1988). Ecological balance is more difficult to achieve in a highly modified agroecosystem, for example, a large-scale monoculture farm. In ecological terms, an agroecosystem is similar to an early successional community (Price and Waldbauer 1994). From planting through harvesting, an annual cropping system is similar to an early successional community that is characterized by simple community structure, low species diversity, low complexity and short-term life cycles. Stability may be difficult to establish in a early successional community. The goal of farming is to maximize the production of a single crop intensively, therefore, a pest population may build up quickly with such abundant food resources. A pest-management approach should try to develop an ecosystem that emulates later stages of succession to attain as much stability as possible (Price and Waldbauer 1994). For example, establishing refuges to maintain populations of natural enemies in a field is an application of this principle. In spite of the intrinsic growth potential of pest populations, the population growth of an insect pest is determined by two opposing external forces: 1) forces that favor the growth such as available and susceptible food resources, favorable weather, lack of natural enemies, and competition and 2) forces that limit the population growth such as resistant plants, natural enemies, lack of shelter and cultural practices that interrupt life cycles of pests. IPM strategies are intelligently used to maximize the force

and strength of natural controls that limit the population growth in an environmentally friendly matter. In this way, the population of insect pests can be maintained below the economic injury level in a sustainable agricultural ecosystem.

IPM is the intelligent application and implementation of sound ecological principles to achieve economic profit and environmental and social benefits. Agriculture creates relatively simple ecosystems and many characteristics of agroecosystems depend on human decision. A farmer can decide when to start the succession (planting), when to terminate the succession (harvesting), and how to apply different cultural practices. Good decision making will maximize the forces that regulate pest populations by altering one or more of the processes or interactions that take place in the agroecosystem. A successful IPM program must consider economic, environmental and social impacts. The necessary elements for a successful IPM program can be summarized as follows. First, IPM should be developed on the basis of sound ecological principles. Agricultural ecosystems can be dissected into multiple interrelated components such as soil, crops, fertilizer, insects, weeds, pesticides, etc. Studies of these compounds provide quantitative information about the characteristics of each component and the relationships between them. Second, IPM tactics must easily fit into the whole context of production. The net benefit of a system should be improved by changing one or more components. A strategy may not be useful if it conflicts with other management methods or it is too complex to be implemented. The final system must be designed for greatest simplicity for the producer (Eigenbrode and Trumble 1994). Third, a balanced value must be achieved by the implementation of an IPM program. Economic profit, environmental impact, and societal factors must be weighed. In most cases, economic profit is foremost because the

agricultural industry is driven by a competitive global market. Environmental impact and social factors are also very important because of public concern about environmental quality and safety issues such as the health effects of pesticide and genetically modified organisms.

### **Host Plant Resistance in IPM**

Host plant resistance is an important tool in most IPM programs and should be the centerpiece of integrated approaches to pest management (Wiseman 1999). The theoretical framework of host plant resistance research was established by Painter (1951). Painter defined three “functional categories” (Smith 1989) of resistance: non preference, antibiosis, and tolerance. Applied entomologists have used this trichotomy as the theoretical foundation for their research for over 50 years, although the term “antixenosis” has largely replaced “non preference” (Kogan and Ortman 1978).

Antixenosis refers to an effect on pest behavior that causes an insect to select an alternate host plant. Antibiosis refers to adverse effects on the biology and physiology of insects and is often manifested by increases in mortality, and decreases in reproduction and weight gain of affected insects. Tolerance refers to the ability of a plant to compensate the injury caused by an insect allowing the tolerant plant to maintain growth or produce high yields despite injury.

The use of host plant resistance to insects has had some outstanding successes. The most outstanding early success was the control of grape phylloxera, *Phylloxera vitifoliae* (Fitch) in Europe using a highly resistant grape vine (*Vitis* spp.) from the U.S.A (Panda 1979). In 1880, the entire grape industry in France was devastated by *P.vitifoliae* and no effective method of control was available. The introduced resistant variety replaced the

susceptible grape variety in vineyards and saved the entire industry for large than 100 years (Smith 1992). Another significant example is the release of wheat varieties resistant to the Hessian Fly, *Mayetiola destructor* (Say). This pest caused tremendous economic loss in the mid west U.S.A. Extensive use of a resistant variety kept Hessian fly densities below the economic injury level for over 50 years and brought about tremendous economic returns (Wiseman 1999). During the last century, resistant crops have played a significant role in pest management world wide. Compared to other control methods, the use of host plant resistance has the highest return value with \$300 return for each \$1 invested (Luginbill 1969). In the United States alone, the economic benefits of the use of insect-resistant alfalfa, barley, corn, sorghum, and wheat cultivars were estimated to be over \$1.4 billion annually (Smith et al. 1994). In the last decade, transgenic crops with genes conferring resistance to insects (Bt cotton, soybean and corn) has brought additional economic benefits to American agriculture (Smith et al. 1994).

Incorporating host plant resistance into an IPM program has the following advantages:

1) It generally targets a specific group of insects and seldom impacts non-target or beneficial organisms (Kogan 1994). For example, the widely-used Bt crops express the Delta endotoxin gene from the spore-form of the bacteria *Bacillus thuringiensis* (Bt). The endotoxin is primarily active against Lepidopteran, and beneficial insects such as parasitoids and predators are not harmed by the Bt toxin (English and Slatin 1992). The specificity of host plant resistance is essential for its application in IPM. 2) Plant resistance is generally compatible with other control tactics. 3) Plant resistance is easily adopted by growers and incorporated into normal management programs because it entails little or no extra cost. 4) Use of host plant resistance is sustainable if it is

implemented in such a way as to mitigate the development of biotypes. 5) The effect of resistant plants on populations of the target insects is cumulative. Pests may not be excluded completely by use of host plant resistant alone, but use of resistant varieties over several years may result in the decline of pest populations.

The level of resistance found in crop plants ranges from low levels of resistance to near-total immunity. Accordingly, host plant resistance has been used as a primary control or as an adjunct to other control methods (Wiseman 1994). Use of resistant plants alone rarely gives complete control of insect pests without the use of supplemental tactics. The well-known cases of primary control (e.g. Hessian Fly, *Mayetiola destructor* (Say) in wheat, grape phylloxera, *Phylloxera vitifoliae* (Fitch) in grape and Brown planthopper *Nilaparvata lugens* in rice) are exceptional. In most cases, the use of plant resistance provides partial control of pest populations.

The effects of the three categories of resistance on insect population dynamics differ from each other. The impact of host plant resistance on populations of insect pests can be categorized into two groups: plant resistance that results in decline of pest populations (antibiosis and antixenosis ) and plant resistance that reduces yield losses without affecting pest populations (tolerance) (Kennedy et al. 1987). Antibiosis causes physiological disorders in insect pests. As a consequence, the rate of population increase declines because of reduced reproduction, growth and survival. Antixenotic plant traits reduce the initial number of insect colonizers for each generation, but the rate of population increase for successful colonizers is not decreased. For both cases, resistant varieties prolong and delay the time needed for a colonizer to reach the economic threshold compared to susceptible varieties (Kennedy et al. 1987). For tolerance, rates of



population increase of insect pests are not affected, Instead, the impact of an insect feeding on yield or crop growth and development is reduced.

Evolution of insects pests in response to mortality factors makes reliance on single control strategies unsustainable in the long term. Generally, insects pests are r-strategists characterized by rapid adaptation to changing environments (Raven and Johnson 1989). High level host plant resistance, especially antibiosis and antixenosis, create high selection pressure on pest populations similar to that created by the use of insecticide. For example, Bt resistant strains of some Lepidopteran pests have already begun to develop on Bt crops (Tabashnik 1994). An extreme argument against sole reliance on a high level of resistance for insect pest control is that “if a control technique is already adequate on its own, there may be an advantage in making it *less* effective if it can be used in combination with other methods ” (van Emden 1991). The essential point of this argument is that the advantage to the use of a combination of strategies is that it decreases the possibility of development of resistant pests. However, few studies have compared the advantages and disadvantage of using host plant resistance as a primary control and using host plant resistance in combination with other strategies. It may not be necessary to use low level resistance if measures are taken to prevent the development of biotypes, such as setting up refuges.

However, in most cases, host plant resistance provides an intermediate level of resistance. Therefore, it is important to understand how low to moderate levels of resistance function within an IPM program. Low to moderate levels of resistance can play a significant role in the context of an IPM program. The effects of low levels of resistance are cumulative and may be sufficient for long-term control. These types of

cumulative effects are likely to be most significant for species that invade crops in low numbers early in the season and build up in number gradually over several generations before reaching damaging levels (Kennedy et al. 1987). Examples of such species are aphids, white flies and mites. Low levels of resistance have continuous and accumulated effects on the population growth of resident insects over generations. For instance, the accumulated effect of low levels of resistance to the corn earworm in maize reduced the number of corn earworm generations and reduced the size of the corn earworm population by 80% in contrast to populations in a susceptible maize variety (Barry et al. 1999). The cumulative effects may be adequate to maintain the population below economic thresholds.

Another major contribution of low to moderate levels of resistance to an IPM program is that low to moderate levels of resistance may increase the effectiveness of other control tactics. In some cases, using resistance enhances the function and efficiency of natural enemies. Combining a parasite and resistance in corn reduced the growth of fall armyworm populations in an additive fashion (Isenhour and Wiseman 1989). Use of a resistant cultivar of sweet corn increased the functional response of *Orius insidiosus*, a predator of fall armyworm and corn earworm larvae, because the period of larval growth was prolonged (Isenhour et al. 1989). The use of a resistant plants may also enhance the efficacy of insecticides. Toxicity of an insecticide is often a function of the body weight of insects. Efficiency of an insecticide may be increased because of reduced body weight caused by antibiosis. For example, white backed planthoppers *Sogatella furcifera* (Horvath) and brown planthoppers feeding on resistant or moderately resistant rice

cultivars were killed with a lower rate of insecticide than those feeding on susceptible cultivars (Heinrichs 1994).

Use of resistant plants may also reduce the number of insecticide applications needed because total insect populations are smaller or population growth is slower. Reduced use of insecticides can have a number of benefits: preventing outbreaks of secondary pests, mitigating impacts on the environment, slowing down the development of insecticide resistance, and preserving natural enemies. The use of sorghum hybrids resistant to greenbugs, *Schizaphis graminum* (Rondani) biotype C, permitted the use of extremely low rates of insecticide (Cate et al. 1973). As an additional effect, the low rate of insecticide preserved populations of natural enemies that prevented the resurgence of greenbug populations.

Management programs can be simplified using moderate levels of resistance (Kennedy et al. 1987). A resistant crop may prevent a resident pest from developing high populations late in the season, eliminating the need for other control measures. If the crop possesses resistance to multiple pests, arthropod management will be greatly simplified. Moreover, combining plant resistance with cultural practices can focus insect populations in time and space and reduce the area to be treated with insecticides. An outstanding example is that of Newsom and Herzog (1977) in Louisiana using a mix of resistant and susceptible soybean as a cultural practice to trap the beetle on susceptible varieties (Hokkanen 1991). Insecticide applications were then made only on the areas planted with the susceptible crop.

The role of tolerance in an IPM program differs from the role of antibiosis or antixenosis because the pest population is not limited. However, the economic injury

level is elevated, allowing higher populations to exist in the field without yield loss. This reduces the need for insecticide. For example, Wiseman et al. (1973) used a tolerant sweet corn hybrid combined with reduced rates and applications of insecticide to control earworm. In some cases, higher insect pest populations create good conditions for microbial control because high insect pest population densities are beneficial to the development and maintenance of pathogen epidemics (Quisenberry and Schotzko 1994). Another advantage of tolerance over antixenosis and antibiosis is that there is no risk of biotype development. Thus, the use of tolerant varieties may be more sustainable than use of antixenosis and antibiosis if the increasing pest population can be prevented from exceeding the economic injury level by other control tactics.

Interactions between host plant resistance and other control strategies are case-dependent and can be independent, synergistic or antagonistic. Whether the integrated effect is positive or negative depends on the particular crop and complex of pest and beneficial species. In some cases, the interaction between host plant resistance, biological control, and insecticide can be antagonistic. High levels of the allelochemical gossypol in resistant cotton cultivars increases expression of the N-demethylase enzyme in the cotton leafworm, *Spodoptera littoralis*, which detoxifies insecticides (El-Sebae et al. 1981). Resistant tomatoes with high levels of tomatine adversely affected a parasite, *Hyposoter exiguae* (Viereck), of the tomato fruitworm *Helicoverpa zea* (Boddie) (Campbell and Duffey 1979). On the basis of these facts, the assumption of compatibility of host plant resistance with other IPM components must be evaluated for each agricultural system.

Not only can host plant resistance influence the effectiveness of other control tactics, but the effectiveness of host plant resistance can also be influenced by numerous environmental factors and cultural practices. When rates of nitrogen are increased, it is generally thought that foliar resistance will be negated by the increased nutritional status of plants, leading to increases in insect pest populations and damage. This is often true, although there are exceptions (Scriber 1984). Barbour et al. (1991) provided a typical example. The resistance response of tomatoes to increases of fertilizer level depended on varieties. Some varieties expressed increased resistance while other varieties showed decreases in resistance level. Another important environmental factor associated with cultural practices is water stress. Traditionally, water stress was thought to cause plants to be more susceptible to herbivores because drought-stressed plants are generally more sensitive to damage and contain more nutrients (Schoonhoven et al. 1998). However, there are many cases in which water stress causes adverse or mixed effects on insects. In addition, changing plant density has been shown to affect tolerance (Trumble et al. 1993, Strauss and Agrawal 1999). Therefore, manipulating environmental conditions by changing cultural practices will have a profound impact on the expression of host plant resistance.

### **IPM of Rice Water Weevil**

Rice, *Oryza sativa*, is the primary cereal for more than two billion people in Asia and for hundreds of millions people in other parts of the world. The rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, is the most destructive insect pest of rice in the United States, and has recently assumed global importance due to its introduction into rice-producing regions of Asia (Heinrichs and Quisenberry 1999). Both adults and larvae

of *L. oryzaophilus* feed on rice, but it is generally the larval stage that causes yield losses (Way 1990). Adults feed on young rice leaves in flooded or unflooded rice fields, leaving feeding scars parallel to the venation of leaves. Oviposition commences when fields are flooded (Everett and Trahan 1967). Larvae may feed on or in leaves for a short period of time after eclosion, but eventually move down to the roots. Larvae feed on or in roots, progressing through four instars and a pupal stage (Cave et al. 1983; Smith 1983). Root pruning by larvae results in reductions in vegetative growth, tillering, grain number, and grain weight (Zou et al. 2004). Yield losses caused by *L. oryzaophilus* in Louisiana, where this insect is a particularly severe pest, typically exceed 10%, and can reach 25% or more under severe infestations (Smith 1983, Anonymous 1994, Stout et al. 2000).

Louisiana rice producers currently rely almost exclusively on insecticides to manage the rice water weevil. The carbamate insecticide carbofuran (Furadan<sup>®</sup>, FMC) was used to manage the rice water weevil until the late 1990's, when the registration for use of this insecticide in rice was cancelled. Following this regulatory action, four insecticides were registered for use against the rice water weevil: Icon<sup>®</sup> (fipronil, Bayer), Karate<sup>®</sup> (lambda-cyhalothrin, Syngenta), Fury<sup>®</sup> (zeta-cypermethrin, FMC) and Dimilin<sup>®</sup> (diflubenzuron, Crompton). Concerns about the toxicity of the newly-registered insecticides to non-target organisms and about the long-term sustainability of heavy reliance on insecticides have prompted investigations of alternative strategies for management of the rice water weevil (Stout et al. 2001).

Historically, host plant resistance has played an important role in pest management programs in rice (Heinrichs 1994) throughout the world. According to a report from the International Rice Research Institute (Smith 1994), more than 20 million ha of rice in

Asia were planted with varieties resistant to the brown planthopper *Nilaparvata lugens* and an additional 10 million ha were planted with varieties resistant to various other insect pests throughout the world. In addition, there are many successful cases of the combined use of resistant cultivars and other tactics such as chemical control, biological control and cultural practices (Smith 1994). Thus, rice varieties with resistance to multiple insect pests have been widely planted and have made a great contribution to global rice yield stability.

In the United States, a collaborative program conducted by USDA and Louisiana State University researchers over the past 30 years has screened more than 8000 rice lines for antibiosis and antixenosis to the rice water weevil (Heinrichs and Quisenberry 1999). Despite this tremendous effort to discover resistant rice lines, no lines possessing high levels of resistance have been found. However, several lines (e.g. PI321264 and PI321278) that consistently supported lower densities of weevil larvae than susceptible varieties were identified. Resistance in these lines was tentatively classified as antixenosis or antibiosis (Smith and Robinson 1984, Robinson et al. 1988, N'Guessan and Quisenberry 1994, Rice et al. 1994). In addition, Smith and Robinson (1982) evaluated more than 100 commercial rice cultivars for resistance and several varieties were found with low levels of resistance. Some of the varieties resistant to the rice water weevil were also resistant to other pests. None of these varieties with low levels of resistance are widely planted now (Heinrichs and Quisenberry 1999, Way 1990).

Tolerance of rice plants to the rice water weevil was also investigated by previous entomologists in Louisiana and several lines were found with tolerance (Gifford and Trahan 1975, 1976, N'Guessan et al. 1994). In the 1980s, Robinson and co-workers

(Robinson and Smith 1984, Robinson et al. 1988) characterized tolerance in several lines of rice (e.g., WC1711) from various sources. These lines were later used as germplasm for the development of several cultivars that are planted widely now (e.g., “Cypress”; S. D. Linscombe, LSU rice breeder, personal communication). In 1990s, N’Guessan et al. (1994) and N’Guessan and Quisenberry (1994) identified several rice lines as having greater tolerance to rice water weevil injury than susceptible standards. Tolerance in these latter lines was manifested by an enhanced ability to recover from root pruning and production of high yields even under high levels of infestation.

Recent work conducted by Stout and colleagues with modern varieties and with lines originally screened by Robinson and co-workers (Smith and Robinson 1982, Smith and Robinson 1984, Heinrichs and Quisenberry 1999) has confirmed that some of these lines possess a low level of resistance (antixenosis or antibiosis) to the rice water weevil. In a greenhouse study, lines PI 321264 and PI 321278 harbored lower number of eggs and first instar larvae than commercial lines Mars and Cypress (Stout and Riggio 1998). In field experiments, the long-grain commercial cultivar “Jefferson” had significantly lower numbers of larvae on its roots than several other commercial cultivars indicating that it possesses antixenosis or antibiosis (Stout et al. 2001). In a recent greenhouse experiment, antixenosis was detected in several breeding lines (Stout and Riggio 2002). These lines may be useful as germplasm for developing resistant varieties in a breeding program. Levels of resistance found in this experiment were greater than were found in previous studies. The lines PI 319512 and PI 321264 showed 17% or greater reductions in egg densities compared to the most resistant commercial variety, “Jefferson”, PI 319512, PI 321264, PI 321278 and PI 321310 showed a 20% or greater reduction in larval densities

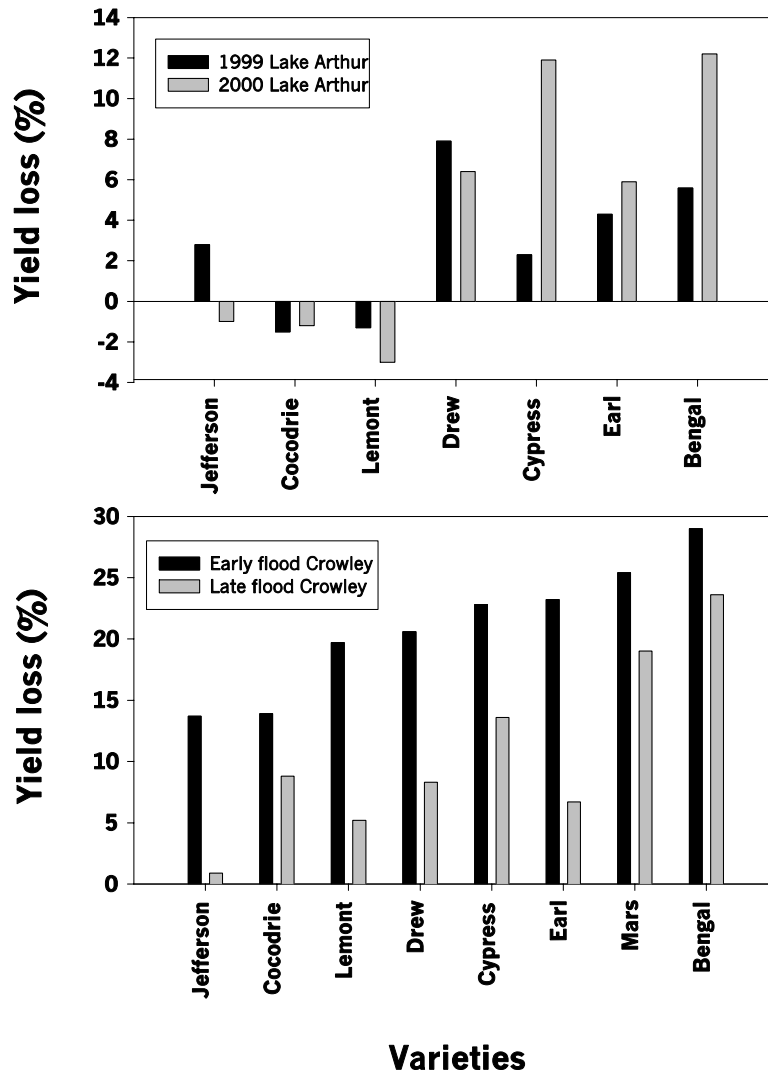


(Stout and Riggio 2002). Although these experiments were primarily designed to detect antixenosis, high rates of mortality from egg to larval stage were found in line PI 319512, perhaps indicating antibiosis. The identification of antixenotic lines and additional highly susceptible lines may facilitate the characterization of biochemical or morphological mechanisms of resistance to the rice water weevil in these lines (Stout and Riggio 2002). A marker-assisted breeding program has been initiated to incorporate antixenosis and antibiosis into commercially viable varieties.

Although development of lines with resistance may represent a long-term solution, the use of commercial varieties with tolerance to the rice water weevil may be a more practical solution for IPM of the rice water weevil at the present time. Modern commercial rice varieties and breeding lines were recently screened for tolerance. A preliminary field experiment with line WC1711 confirmed that this line is more tolerant to rice water weevil than the susceptible cultivar “Mars” (Stout and Rice unpublished data). Several varieties were found with low to moderate levels of tolerance to feeding by weevil larva. in two years of field studies at Lake Arthur (Vermilion Parish, LA) and Crowley (Stout et al. 2001). The Lake Arthur studies in 1999 and 2000 showed that the long-grain cultivars “Cocodrie” and “Lemont” were more tolerant of weevil injury than other cultivars (Fig. 1.1, Stout et al. 2001). The average yield loss for “Cocodrie” was about 1/10 of that of the susceptible variety, “Bengal”, subjected to similar larval infestation level. Medium grain varieties growing in Louisiana appear to be less resistant and tolerant than long grain rice cultivars. It is not clear whether this is due to grain size per se or due to the fact that all medium grains grown in Louisiana have similar pedigrees (Linscombe et al. 1992).

Recent research also suggests that cultural practices and host plant resistance for management of the rice water weevil are compatible. Several studies have demonstrated that short delays in flooding can reduce yield losses from the rice water weevil (Rice et al. 1999, Stout et al. 2001). This strategy is effective because oviposition does not occur until fields are flooded (Everett and Trahan, 1967, Stout et al., 2002a) and older plants are more tolerant of larval feeding than younger plants (Wu and Wilson 1997, Stout et al. 2002b). Theoretically, the use of rice varieties tolerant of the rice water weevil is compatible with delayed flooding, and both strategies are probably compatible with applications of insecticide. Evidence from Stout et al. (2001) suggest that the tolerance exhibited by some varieties was accentuated in a delayed-flood regime (Fig. 1.1). For example, yield losses from plots of “Jefferson”, one of the most tolerant varieties available, averaged 13.7% under an early-flood regime but only 0.9% under a delayed-flood regime. This comparison suggests that delaying flood may create conditions more suitable for the expression of tolerance. Incorporating tolerant rice varieties and delayed flooding into the management program for the rice water weevil may allow reductions in rates or numbers of insecticide applications without compromising yield. If this hypothesis is true, multiple benefits could be obtained from this diversified program. Reduced insecticide will reduce costs to growers and will also reduce environmental costs associated with insecticide containment. Even if insecticide rates can’t be reduced, growers may benefit from increased yields resulting from greater tolerance.

No comprehensive theory has been developed regarding the role of host plant resistance in integrated pest management programs; rather, the outcome of integrating systems. Information on the use of low levels of resistance to the rice water weevil in a



**Figure 1.1** Yield losses of eight varieties from untreated plots compared to Icon treated plots in Lake Arthur, LA and in Crowley, LA. Experiments in Crowley were subjected to early and delayed flooding regime (Stout et al. 2001).

management program for this insect will contribute valuable information to literature on the use of host plant resistance in IPM. In addition, information about root-feeding insects such as the rice water weevil is lacking compared to above ground insect pests. The rice-rice water weevil system provides a unique system to study the role of low levels of host plant resistance in IPM. In addition, evaluation of the potential role of low levels host plant resistance in the weevil management program will provide basic information about the future development of IPM for the rice water weevil.

In a recent review of IPM (Way and van Emden 2000) in which different methodologies of IPM research were evaluated, Way and van Emden concluded that sound empirical investigations of applied ecology are the most valuable approaches for improving IPM programs. Since the 1970s, it has been emphasized that improvement in IPM depends on a sophisticated and in-depth mathematical understanding of the structure and dynamics of agroecosystems (Kogan 1998). The publication, “Ecological Based Pest Management” (National Research Council 1996) provided a theoretical framework for pest management in the future. Theoretically, the functions of an ecological system can be predicted if all interacting components were mathematically described. On the basis of this premise, massive efforts were made to study and model fundamental processes of agroecosystems. However, despite a large investment of human and monetary resources, improvement of IPM decision-making was marginal (Way and van Emden 2000). One reason for this is that the complexity of ecosystems makes it almost impossible to obtain a comprehensive understanding because of limited human and funding resources. For example, irrigated rice in Asia has potentially 9000 trophic links between 645 taxa which vary in time and space (Cohen et al. 1994). Undoubtedly, sound IPM should be based on

a thorough understanding of agroecosystems. Instead of trying to dissect the complicated system into every single piece, experimental approaches with hands-on field testing of hypotheses is more productive and valuable for IPM (Way and van Emden 2000). The performance of management tactics is evaluated by conducting field experiments in real situations. However, modeling approaches and experimental approaches are not exclusive of one another; instead, they are complementary. The experimental approach is based on a fundamental understanding of ecology and, meanwhile, prediction from ecological models need to be verified by experimental testing. Unbalanced emphasis has been given to the modeling approach, and priority should be given to application of the right kinds of applied ecological and experimental field studies in real situations (Way and van Emden 2000). For this study of IPM for rice water weevil control, a majority of effort was focused on experimental testing of hypotheses in small rice plots. In addition, some modeling work will be done to aid the study.

### **Objectives**

The objective of this research was to evaluate the effects and roles of low to moderate levels of resistance, particularly tolerance, in an IPM program for the rice water weevil, *L. oryzaophilus*. The overall hypothesis is that the use of plants that possess low to moderate levels of host plant resistance is compatible with other control tactics such as delayed flood and insecticide applications, and that similar yields can be obtained by using integrated tactics of delayed flood, tolerant varieties and reduced insecticide application. An empirical approach aided by modeling work was used. The objectives

were:

- i. To test the consistence of expression of tolerance to *L. oryzaophilus* feeding in several rice varieties over multiple years (Chapter 2).
- ii. To characterize varietal difference in tolerance to *L. oryzaophilus* injury in an effort to understand the mechanism of yield loss (Chapter 3).
- iii. To characterize the relationship between rice yields and densities of *L. oryzaophilus* larvae using two varieties under early and delayed flooding regimes (Chapter 4).
- iv. Use a degree-day model to predict the emergence of adults of *L. oryzaophilus* from overwintering sites (Chapter 5).
- v. To quantify the development of *L. oryzaophilus* on rice varieties with different levels of resistance (Chapter 5).

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## **CHAPTER 2**

### **CONSISTENCY OF EXPRESSION OF RICE PLANT TOLERANCE TO RICE WATER WEEVIL FEEDING OVER MULTIPLE YEARS**

#### **Introduction**

Prior experiments have shown that rice varieties differ in resistance and tolerance to infestation by the rice water weevil (Stout et al. 2001). Host plant resistance is influenced by environmental factors such as soil type, cultural practices and pest pressure levels (Smith 1989) and thus levels of resistance expressed by a given genotype may differ from year to year. “Cocodrie” and “Bengal” are two commercial varieties commonly grown in Louisiana. “Cocodrie” was more tolerant of weevil feeding than “Bengal” whereas the two varieties were equally susceptible to infestation over two years in a previous study (Stout et al. 2001). There have been no systematic studies over multiple years to document the difference in tolerance of these two varieties. If tolerance is highly variable, the potential of integrating tolerance into an IPM program is low. The purpose of this study is to confirm differences in tolerance found in previous studies (Stout et al. 2001) to determine the consistency of expression of tolerance from year to year.

#### **Material and Methods**

Experiments were conducted at the Louisiana State University Rice Research Station (Crowley, Acadia Parish, LA) in 2001, 2002 and 2003. The soil type was a silt loam (fine, montmorillonitic, thermic typic albaqualf). Rice was drill-seeded on 10 April (2001), 3 May (2002) and 17 April (2003) at a rate of 100 kg ha<sup>-1</sup> (2001, 2002) or 124 kg ha<sup>-1</sup> (2003). Plot size was 1.5 meter by 6 meter. Permanent flood was applied on 11 May (2001), 31 May (2002) and 15 May (2003). Total nitrogen fertilization rate was 135 kg

ha<sup>-1</sup>, with the majority of fertilizer applied before flooding. Fields were harvested on 22 August (2001), 9 September (2002) and 27 September (2003). Other agronomic practices used were typical of those used in southwest Louisiana.

The experimental design in 2001 and 2002 was a randomized complete block with 4 treatments and 4 replications. The four treatments were factorial combinations of the varieties 'Bengal' and 'Cocodrie' and two insecticide treatments. The two insecticide treatments were 1) exclusion of insects using Icon<sup>®</sup> (fipronil, Bayer) at a rate of 0.056 kg AI/ha (active ingredient per hectare) and 2) no exclusion of insects (no insecticide). In 2003, the experimental design was a randomized complete block with 6 treatments and 2 replications. The six treatments were factorial combinations of two varieties and three insecticide treatments. The three insecticide treatments were 1) exclusion of insects using fipronil at a rate of 0.045 kg AI/ha, 2) one application of Karate<sup>®</sup> (lambda-cyhalothrin, Syngenta) at a rate of 0.034 kg AI/ha and 3) no exclusion of insects (no insecticide). Although the rate of fipronil used in 2003 differed from the rate used in the previous two years, both rates can be considered exclusion treatments since fipronil rates in excess of 0.045 kg AI/ha are high enough to exclude the great majority of weevil larvae. The lambda-cyhalothrin treatment was considered a reduced insecticide treatment because the rate of lambda-cyhalothrin used (0.034 kg AI/ha) was 24% lower than the rate of fipronil used (0.045 kg AI/ha).

Densities of *L. oryzaophilus* larvae and pupae were determined by taking root/soil core samples from plots (Everett 1963). The core sampler was a metal cylinder with a diameter of 9.2 cm and a depth of 7.6 cm. For each plot, three or four cores were taken from the interior of plots at 1 and 12 Jun (2001), 28 Jun (2002) and 24 Jun (2003). Cores

were placed into 40 mesh screen sieve buckets and soil was washed from roots. Buckets were placed into basins with salt water, and larvae and pupae were counted as they floated to the water surface. Immature densities were averaged for each plot.

Data were analyzed using a mixed model in SAS (Littell et al., 1996). The model considers the blocking factor (replicates) as a random effect and variety and year as fixed effects. Because the intent of this study was to examine the consistency of varietal effects over multiple years, and because similar experimental designs were employed in all three experiments, data from all three years were analyzed together. The presence of a significant year x variety effect in the analysis would indicate lack of consistency of varietal effects. In addition, yield losses for each year were compared between varieties using CONTRAST statements in SAS. All data analyses were conducted using PROC MIXED of SAS (Littell et al. 1996). Yield loss was calculated using the formula:  $1 - (\text{yld}_{p,k} / \text{yield}_{I,k})$ , where  $\text{yld}_{p,k}$  represents yield from an untreated plot from block k and  $\text{yield}_{I,k}$  represents yield from a fipronil-treated plot in block k.

## **Results**

Larval and pupal densities were significantly reduced in fipronil-treated plots in all three years (Table 2.1). In all cases, the reduction in immature densities of rice water weevils in fipronil-treated plots (70% to 90% reduction) was sufficient to consider the two treatments as “infested” and “not infested”. Yield losses were lower from plots of “Cocodrie” than from plots of “Bengal” in all three years (Table 2.1). Although differences in yield losses between the two varieties were significant in only one of three years, the lack of interaction between year and variety in the statistical analysis (Table 2.2) indicates that the pattern was consistent over all three years. There was no

significant difference in yield between fromf plots treated with fipronil and plots treated with one application of lambda-cyhalothrin in 2003 (Table 2.1).

### **Discussion**

“Cocodrie” appears to be more tolerant of rice water weevil feeding than “Bengal”. This difference was expressed consistently over the three years of this study as well as in two years of a previous study (Stout et al., 2001). The experiments were conducted at different sites at the Rice Research Station each year. Soil conditions and fertilities undoubtedly varied among experiments. In addition, infestation pressure of rice water weevils fluctuated from year to year. Thus, the tolerance of the commercial variety “Cocodrie” is consistent for general cultural conditions. Results from Karate-treated plots in 2003 suggest that the use of a more tolerant variety combined with a single application of Karate (reduced insecticide) may be sufficient to adequately manage rice water weevils. Using tolerance as a management tactics is a reliable source for IPM programs managing rice water weevil.

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**Table 2. 1.** Densities of rice water weevil immature and yields of four rice varieties in 2001, 2002 and 2003 (means  $\pm$  S.E.) from plots treated with different levels of insecticide (untreated control and fipronil)

Year Variety	Densities of immature weevil (larvae + pupae per core)		Yield (Kg/ha) <sup>1</sup>		Yield loss (%) <sup>3</sup>
	UTC <sup>2</sup>	fipronil	UTC	fipronil	
2001	UTC <sup>2</sup>	fipronil	UTC	fipronil	
Cocodrie	14.9 $\pm$ 1.9	3.0 $\pm$ 1.0	6159.1 $\pm$ 291.7 <sup>a</sup>	7307.0 $\pm$ 125.1 <sup>b</sup>	15.5 $\pm$ 4.9 <sup>c</sup>
Bengal	18.6 $\pm$ 2.6	2.0 $\pm$ 1.4	6566.0 $\pm$ 403.2 <sup>a</sup>	8175.9 $\pm$ 148.2 <sup>b</sup>	19.7 $\pm$ 4.8 <sup>c</sup>
2002	UTC	fipronil	UTC	fipronil	
Cocodrie	43.3 $\pm$ 7.5	6.0 $\pm$ 0.4	7649.7 $\pm$ 288.9 <sup>a</sup>	8567.9 $\pm$ 85.3 <sup>b</sup>	10.7 $\pm$ 2.1 <sup>c</sup>
Bengal	46.5 $\pm$ 7.6	13.3 $\pm$ 2.4	5634.4 $\pm$ 485.3 <sup>a</sup>	8618.4 $\pm$ 152.4 <sup>b</sup>	34.8 $\pm$ 4.5 <sup>d</sup>
2003	UTC	fipronil	UTC	fipronil	
Cocodrie	15.1 $\pm$ 1.6	2.4 $\pm$ 0.5	5305.4 $\pm$ 245.2 <sup>a</sup>	6986.4 $\pm$ 103.1 <sup>b</sup>	24.1 $\pm$ 4.6 <sup>c</sup>
Bengal	18.1 $\pm$ 2.4	2.9 $\pm$ 0.2	4694.3 $\pm$ 181.8 <sup>a</sup>	6735.3 $\pm$ 143.8 <sup>b</sup>	30.1 $\pm$ 7.6 <sup>c</sup>
2003	lambda-cyhalothrin		lambda-cyhalothrin		
Cocodrie	5.6 $\pm$ 0.9		6783.0 $\pm$ 174.4 <sup>b</sup>		2.9 $\pm$ 0.1 <sup>c</sup>
Bengal	4.9 $\pm$ 1.0		6208.0 $\pm$ 189.4 <sup>b</sup>		7.8 $\pm$ 6.8 <sup>c</sup>

<sup>1</sup>Means in the same column followed by the same letter are not significantly different (CONTRAST).

<sup>2</sup>UTC: Untreated Control.

<sup>3</sup>Yield losses were calculated by comparing yields from fipronil-treated and UTC plots as described in the text. Yield losses from plots treated with lambda-cyhalothrin (2003 experiment) were calculated by comparing yields from lambda-cyhalothrin-treated plots with yields from fipronil-treated plots.



**Table 2. 2.** *P* values for mixed-model analyses for the 2001, 2002 and 2003 experiments.

Year Variety	Yield (Kg/ha) <sup>1</sup>	Yield loss (%) <sup>2</sup>
2001		
Cocodrie	<i>P</i> = 0.0002	<i>P</i> = 0.4886
Bengal	<i>P</i> < 0.0001	
2002		
Cocodrie	<i>P</i> = 0.0304	<i>P</i> = 0.003
Bengal	<i>P</i> < 0.0001	
2003		
Cocodrie	<i>P</i> < 0.0001	<i>P</i> = 0.4781
Bengal	<i>P</i> < 0.0001	
2003 lambda-cyhalothrin		
Cocodrie	<i>P</i> = 0.5879	<i>P</i> = 0.5769
Bengal	<i>P</i> = 0.1946	

Source of variation	Yield losses		
	<i>df</i>	<i>F</i>	<i>P</i>
Variety	1	7.55	0.0189
Year	2	5.43	0.0109
Variety × Year	2	2.01	0.1589

<sup>1</sup>Results of the contrast between untreated plots and insecticide-treated plots for each variety.

<sup>2</sup> Comparison between the two varieties.

## CHAPTER 3

### THE EFFECTS OF FEEDING BY THE RICE WATER WEEVIL ON THE GROWTH AND YIELD COMPONENTS OF RICE

#### Introduction

There is a long history of recording relationships between arthropod pest density and yield loss in agriculture, and these empirical relationships form the bases of economic thresholds (Headley, 1975). Yield losses can result from numerous physiological changes such as reductions in photosynthesis, alteration of source-sink relationships, disruption of plant hormone regulation, etc; however, the physiological mechanisms by which pests affect plant physiology and reduce yields remain poorly understood. The physiology of plants under attack by arthropod pests has often been considered a “black box”, meaning only pest densities and yield components were studied (Peterson & Higley, 2001). The “black box” approach may suffice in the short-term for the development of management strategies, but understanding plant physiological responses to injury is fundamental to the development of long term, sustainable management strategies (Peterson & Higley, 2001). The dearth of studies of physiological responses to injury is particularly true of root-feeding insect pests. Traditionally, root-feeding herbivores have received less attention than herbivores of the aerial portion of plants, and few studies have investigated plant responses to below-ground herbivory (McDonald & Sears, 1991; Gerson, 1996). Substantial research is needed to improve our understanding of the mechanism of yield loss from root-feeding insects.

The rice water weevil, *Lissorhoptus oryzophilus* Kuschel, is the most destructive insect pest of rice *Oryza sativa* L. in the United States (Way, 1990). *L. oryzophilus* is native to North America but has been introduced into Asia, the largest rice-producing

region in the world (Heinrichs & Quisenberry, 1999). Thus, the rice water weevil could become an important insect pest of rice globally. Adults of this insect feed on leaves of rice, producing feeding scars parallel to the venation of rice leaves. Oviposition generally does not commence until rice fields are flooded (Everett & Trahan, 1967). Larvae eclose from eggs, then move into the soil and feed on roots. Larval feeding causes stunted root systems and reduced grain yields (Bowling, 1972). Yield losses typically approach 10% but can exceed 25% under heavy pressure (Smith & Robinson, 1982; Stout et al., 2000).

The relationship between rice water weevil population density and rice grain yield has been quantified in field experiments (Gifford et al., 1975; Barbour et al., 1996). Feeding by *L. oryzaophilus* is known to result in stunted root systems and reduced tillering (Grigarick, 1984; Hesler et al., 2000). However, there has been no formal or comprehensive documentation of the effects of feeding by *L. oryzaophilus* larvae on rice growth, physiology and development. The objective of the research reported here was to document the effects of feeding by the *L. oryzaophilus* on the growth and yield components of rice as a first step toward elucidating physiological mechanisms of yield loss in rice.

## **Material and Methods**

### **General Cultural Practices**

Experiments were conducted in 2000 and 2001 at the LSU AgCenter Rice Research Station (Crowley, Acadia Parish, LA). The soil type was a silt loam (fine, montmorillonitic, thermic typic albaqualf). For the 2000 experiment, rice was drill-seeded on 19 April at a rate of 112 kg ha<sup>-1</sup>. Plot size was 1.5 m by 6 m. Plots were

irrigated on 28 April; permanent flood was applied on 16 May (27 days after planting), when plants were at the 3-5 leaf (early tillering) stage. In 2001, rice was drill-seeded on 23 April at a rate of 40 kg ha<sup>-1</sup>. The seeding rate was reduced compared with that in 2000 to facilitate sampling of individual plants. Plot size was 1.5 m by 9 m. Plots were irrigated on 1 May and flooded on 16 May (24 days after planting) when plants were in the early tillering stage. Total nitrogen fertilization rate was 135 kg ha<sup>-1</sup> in 2000 and 120 kg ha<sup>-1</sup> in 2001, with the majority of fertilizer applied before flooding. Other agronomic practices used were typical of those used in southwest Louisiana.

### **Sampling of Insects**

Densities of *L. oryzaophilus* larvae and pupae were determined by taking root/soil core samples from plots (Everett 1963). The core sampler was a metal cylinder with a diameter of 9.2 cm and a depth of 7.6 cm. For each plot, two cores were taken from the interior of plots. Cores were placed into 40 mesh screen sieve buckets and soil was washed from roots. Buckets were placed into basins with salt water, and larvae and pupae were counted as they floated to the water surface.

### **Experimental Design and Procedures**

#### Single Variety Test in 2000

The intent of the experiments conducted in both 2000 and 2001 was to compare the growth and development of rice plants subjected to natural infestations of rice water weevil larvae with the growth and development of rice plants protected from such infestations. The design of the experiment in 2000 was a randomized complete block with two treatments and five replications. The cultivar “Cocodrie”, a common commercial variety, was used in this experiment. Icon<sup>®</sup> (active ingredient: fipronil;

Aventis Crop Science, Research Triangle Park, NC) was used as a seed treatment to exclude larvae from rice roots in half of the plots. Rice seed was treated at a rate of 0.07 kg AI/ha (active ingredient per hectare). No insecticide treatment was used in the remaining plots. Sampling of larvae was conducted on five dates: 16 days (29 May), 26 days (8 Jun), 38 days (20 Jun), 54 days (6 July) and 76 days (26 July) after permanent flood. Sampling of plants to assess growth and development was conducted four times, when plants were at the late tillering (8 Jun, 26 days after permanent flood), green ring (19 Jun, 37 days after permanent flood), elongating internodes (6 July, 54 days after permanent flood) and heading (25 July, 73 days after permanent flood) stages. Six plants were randomly removed from each plot. After washing soil from roots, the number of tillers on each plant was counted. Plants were then placed inside the greenhouse and air-dried for 72 hours. The dried plants were separated into root and shoot portions. Weights of roots and shoots were measured using an electronic balance (Ohaus Corporation, USA). On 16 August, when plants were mature, six plants were taken from each plot and the yield components were measured. The number of panicles for each plant was counted. Then for all panicles (2-6 panicles/plant), all filled grains were counted. One hundred filled grains were selected from plants of each plot and weighed (Yoshida et al., 1976).

#### Multiple varieties test in 2001

This experiment utilized three commercial varieties, “Cocodrie”, “Cypress”, “Bengal”. These commercial varieties have been shown to differ in their tolerance to rice water weevil injury (Stout et al., 2001). A fourth variety, “XP1003” (an advanced but not yet commercialized hybrid line, RiceTec, Alvin, TX), was also included since it, or a related

variety, potentially will be used by growers in the near future. The experimental design was a randomized complete block with eight treatments and five replicates. Treatments were factorial combinations of the four varieties at two infestation levels (insecticide exclusion and untreated control). Exclusion of weevils was accomplished by treating seeds with fipronil at a rate of 0.07 kg AI/ha. Sampling of larvae was conducted 20, 30 and 40 days after permanent flood. Larval density was measured by taking two core samples per plot. In addition, six plants were periodically taken from each plot for determination of the effects of weevil feeding on plant parameters. Plant sampling was conducted four times during the growing season, when rice plants were at the early tillering (30 May, 14 days after permanent flood), late tillering (11 Jun, 26 days after permanent flood), elongating internodes (28 Jun, 43 days after permanent flood), and heading (19 July, 64 days after permanent flood) stages. Air drying and weighing procedures were the same as in 2000. For each plant, the number of tillers, root biomass and shoot biomass were recorded. Yield components were determined by harvesting a 1.78 m<sup>2</sup> area in the center of plots by hand. The number of panicles contained in this 1.78 m<sup>2</sup> area was counted. Another estimate of panicle density was obtained by harvesting three additional 0.25 m<sup>2</sup> areas from each plot and counting the number of panicles. Panicle density from these four subplots were converted into a panicle number per unit area (panicle number per m<sup>2</sup>). The number of filled grains per panicle was estimated from 10 plants from the 1.78 m<sup>2</sup> subplot. All rice from the 1.78 m<sup>2</sup> subplot was then threshed to determine grain yield. Grain moisture and weight per 1000 grains were determined from threshed rice. Grain moisture was measured using a grain moisture

meter. Weight per thousand filled grains was determined using a grain counter and an electronic balance.

### **Statistical Analyses**

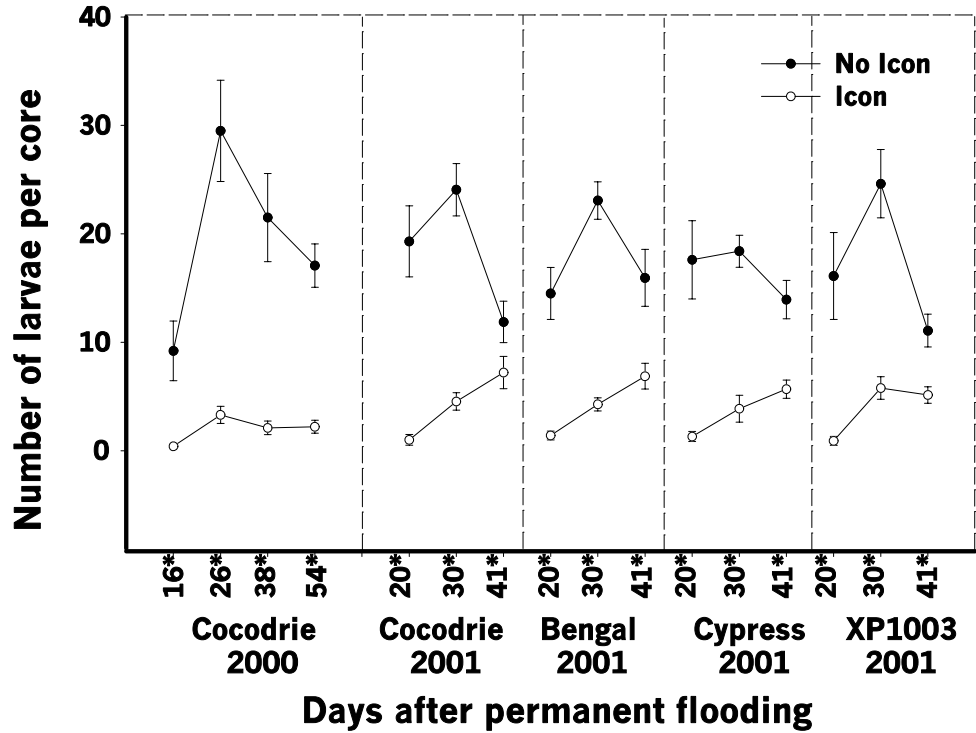
Data on larval density and plant vegetative characteristics (number of tillers, root and shoot biomass) were analyzed using a repeated-measures mixed model in SAS (Littell et al., 1996). This model was suitable for this set of data because data were taken from each experimental unit several times over the course of the growing season. The model considers block (replicate) as a random effect and insecticide treatment, variety, sampling dates and interaction terms as fixed effects.

Yield component data were analysed using a mixed model for a randomized complete block design. The block effect was considered random and treatment and variety effects were considered fixed. Since possible differences in treatment effects between varieties were of interest, planned F tests were used to compare parameters for each variety. This method fit the purpose of the study by partitioning degrees of freedom and sum of squares for treatment effects into meaningful single degrees of freedom and associated sums of squares (Little & Hills, 1978). All data analyses were conducted using PROC MIXED of SAS and planned F tests were conducted using CONTRAST statements (Littell et al., 1996).

## **Results**

### **Single Variety Test in 2000**

Larvae were found associated with roots on all core sampling dates (Fig. 3.1). In the untreated plots, larval numbers reached their maximum levels 26 days after permanent flood and declined thereafter. Use of fipronil excluded the majority of weevils from roots.



**Figure 3.1** Densities of immature rice water weevil (number of larvae and pupae per core  $\pm$  S.E.) on four (2000) and three (2001) sampling dates. An asterisk denotes a significant difference between the fipronil and No fipronil treatments ( $P < 0.05$ , planned F test in Mixed model).



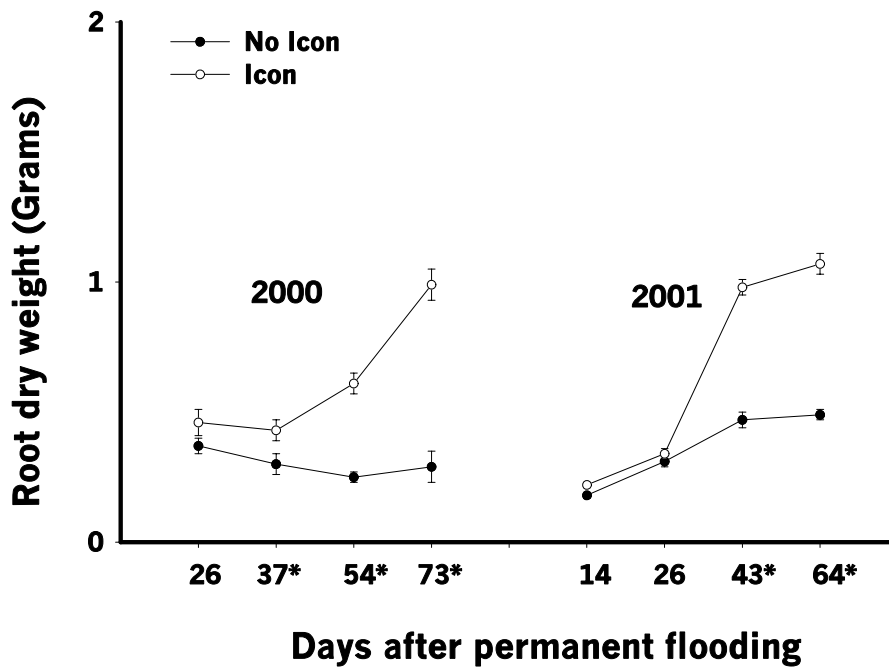
Mean larval density over all sampling dates for untreated plots was 22.7 per core sample, whereas the density was 2.5 per core in fipronil treated plots. Larval density was significantly different between the two treatments ( $F_{1, 20} = 22.10, P < 0.001$ ).

Larval feeding reduced both root and shoot biomass, as evidenced by lower weights for roots and shoots in untreated plots compared to fipronil-treated plots (Fig. 3.2a,  $F_{1, 223} = 124.11, P < 0.001$  for root biomass and Fig. 3.2b,  $F_{1, 223} = 118.47, P < 0.001$  for shoot biomass). Tiller number was also significantly reduced in untreated plots (Fig. 3.3,  $F_{1, 223} = 102.23, P < 0.001$ ). For root biomass, shoot biomass and tiller number, differences between fipronil-treated and untreated plots increased as the growing season progressed, as indicated by a significant interaction between treatment and sampling dates ( $P < 0.001$ ).

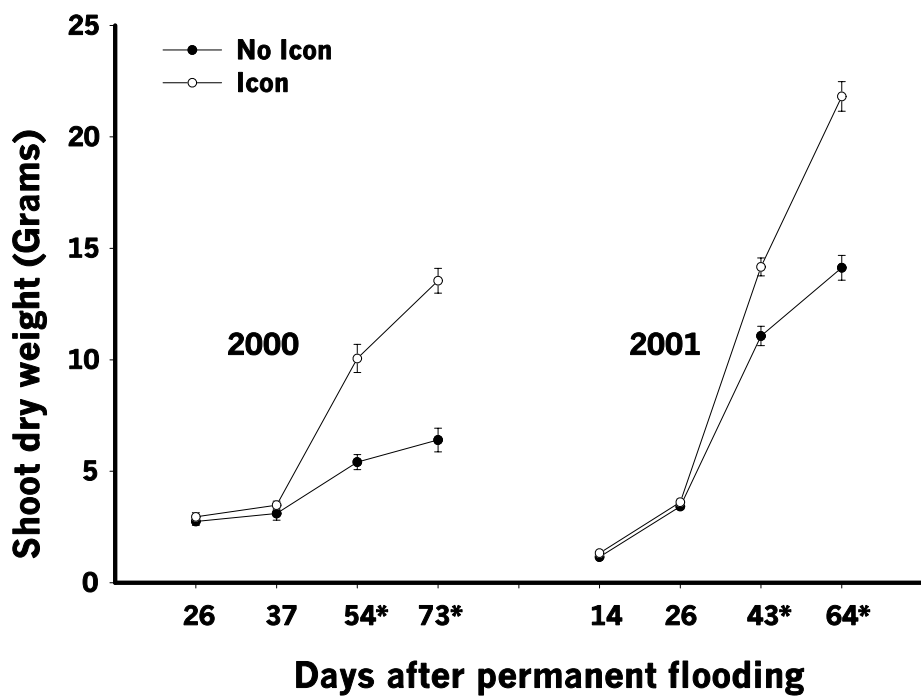
The yield of untreated plots was reduced by about 20% compared to yields in fipronil-treated plots (Table 3.1,  $F_{1, 4} = 14.23, P = 0.02$ ). The number of panicles per plant from untreated plots was about half that in plants from fipronil-treated plots (Table 3.1,  $F_{1, 48} = 91.93, P < 0.001$ ). Mean grain weight was also reduced by weevil injury. The mean weight of 100 grains from untreated plots was 2.39 grams, and the weight of 100 grains from fipronil-treated plots was 2.52 grams (Table 3.1,  $F_{1, 48} = 18.14, P < 0.001$ ). The difference in number of filled grains per panicle between treatments was marginally significant ( $F_{1, 48} = 4.02, P = 0.06$ ).

### **Multiple Varieties Test in 2001**

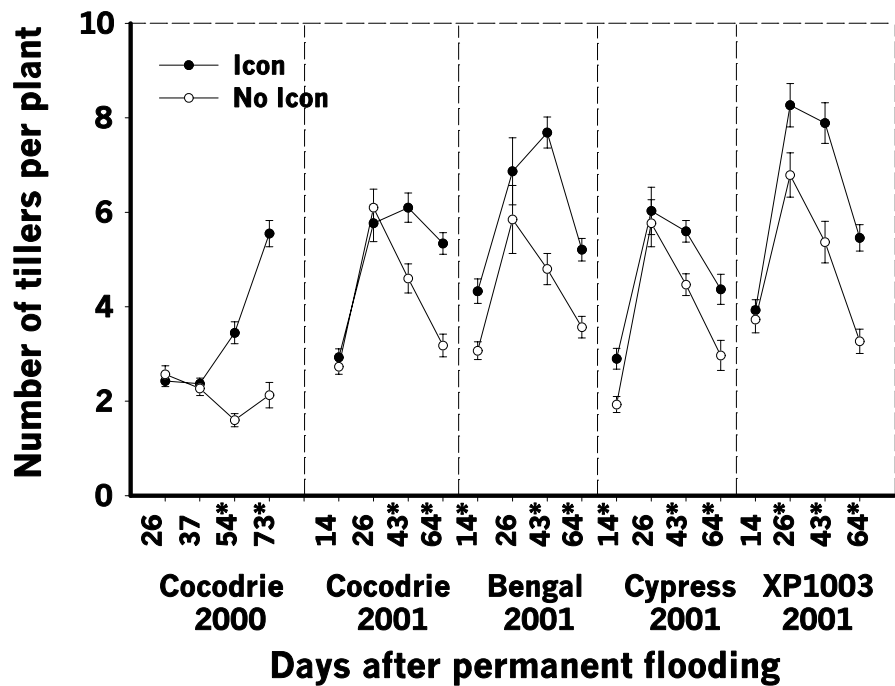
Untreated plots suffered high levels of infestation by rice water larvae, with about 20-30 larvae per core 30 days after permanent flooding. fipronil reduced densities of larvae to



**Figure 3.2a** Biomass of rice plants (dry weights of roots per plant  $\pm$  S.E.) on four samples after permanent flood in 2000 and 2001 experiment. Data in 2001 is the average of four varieties. Data were pooled because effects were similar across varieties. An asterisk denotes a significant difference between fipronil and No fipronil treatments ( $P < 0.05$ , planned F test in Mixed model).



**Figure 3.2b** Biomass of rice plants (dry weights of shoots per plant  $\pm$  S.E.) on four samples after permanent flood in 2000 and 2001 experiment. Data in 2001 is the average of four varieties. Data were pooled because effects were similar across varieties. An asterisk denotes a significant difference between fipronil and No fipronil treatments ( $P < 0.05$ , planned F test in Mixed model).



**Figure 3.3** Tiller densities of rice plant (number of tillers per plant  $\pm$  S.E.) on four sampling dates after permanent flood in 2000 and 2001 experiment. An asterisk denotes a significant difference between fipronil and No fipronil treatments ( $P < 0.05$ , planned F test in Mixed model).

**Table 3.1** Yields and Yield components (mean  $\pm$  S.E.) from plots of rice experimenting natural levels of infestation by *L. oryzaophilus* or protected from infestation using the cultivar Cocodrie, Bengal, Cypress and XP1003 on year 2000 and year 2001.

Variety	Treatment	Adjusted grain Yield* (Kg ha <sup>-1</sup> )	Number of panicles* (plant <sup>-1</sup> (2000), m <sup>-2</sup> (2001))	Number of grains (panicle <sup>-1</sup> )	Grain weight* (g, 100 (2000), 1000 (2001))
Cocodrie 2000	No fipronil	5692.5 $\pm$ 260.7 <sup>a</sup>	2.33 $\pm$ 0.16 <sup>a</sup>	60.4 $\pm$ 4.3 <sup>a</sup>	2.39 $\pm$ 0.03 <sup>a</sup>
	fipronil	7158.4 $\pm$ 511.3 <sup>b</sup>	4.97 $\pm$ 0.21 <sup>b</sup>	69.5 $\pm$ 2.7 <sup>b</sup>	2.52 $\pm$ 0.02 <sup>b</sup>
Bengal 2001	No fipronil	5414.4 $\pm$ 209.9 <sup>a</sup>	329.4 $\pm$ 13.4	84.2 $\pm$ 3.1 <sup>a</sup>	26.54 $\pm$ 0.16 <sup>a</sup>
	fipronil	6517.2 $\pm$ 550.9 <sup>b</sup>	342.2 $\pm$ 14.3	98.8 $\pm$ 3.8 <sup>b</sup>	27.49 $\pm$ 0.33 <sup>b</sup>
Cocodrie 2001	No fipronil	5270.0 $\pm$ 190.7	293.9 $\pm$ 9.7 <sup>a</sup>	90.2 $\pm$ 5.2	24.64 $\pm$ 0.38
	fipronil	5967.7 $\pm$ 316.2	374.8 $\pm$ 15.0 <sup>b</sup>	80.7 $\pm$ 7.4	24.75 $\pm$ 0.36
Cypress 2001	No fipronil	4518.7 $\pm$ 121.6 <sup>a</sup>	371.9 $\pm$ 14.4 <sup>a</sup>	62.6 $\pm$ 5.4 <sup>a</sup>	24.16 $\pm$ 0.08 <sup>a</sup>
	fipronil	5840.4 $\pm$ 648.4 <sup>b</sup>	406.7 $\pm$ 18.5 <sup>b</sup>	75.8 $\pm$ 4.4 <sup>b</sup>	24.97 $\pm$ 0.29 <sup>b</sup>
XP1003 2001	No fipronil	7085.7 $\pm$ 384.3	326.7 $\pm$ 11.6 <sup>a</sup>	101.0 $\pm$ 3.5	25.09 $\pm$ 0.15
	fipronil	7161.0 $\pm$ 495.7	379.7 $\pm$ 15.3 <sup>b</sup>	101.2 $\pm$ 2.8	25.45 $\pm$ 0.09

\* denotes a significant difference across all varieties ( $P < 0.01$ , ANOVA in Mixed model) from fipronil-treated and untreated plots. For each variety, different letter a, b denote there is a significant difference for the mean ( $P < 0.05$ , Planned F test in Mixed model).

about three larvae per core (Fig. 3.1,  $F_{1, 283} = 319.91$ ,  $P < 0.001$ ). Densities of larvae did not differ significantly among varieties ( $F_{3, 283} = 0.13$ ,  $P = 0.94$  for treatment by variety interaction). The effect of treatment varied with sampling date ( $F_{2, 283} = 22.33$ ,  $P < 0.001$  for treatment by time interaction). The interaction may be due to the increase in larval density in fipronil treated plots as the season progressed (Fig. 3.1).

Larval feeding significantly reduced both root and shoot biomass (Fig. 3.2a, root weight:  $F_{1, 899} = 184.41$ ,  $P < 0.001$ ; Fig. 3.2b, shoot weight:  $F_{1, 899} = 118.25$ ,  $P < 0.001$ ). There was an interaction between treatment and sampling dates for both parameters (root weight:  $F_{3, 899} = 46.94$ ,  $P < 0.001$ ; shoot weight:  $F_{3, 899} = 47.77$ ,  $P < 0.001$ ) again indicating that differences in growth parameters increased over the course of the growing season. The number of tillers per plant in untreated plots was significantly lower than that in the treated plots ( $F_{1, 899} = 141.51$ ,  $P < 0.001$ ). The pattern was consistent across all varieties (Fig. 3.3). The difference in tiller number between treated and untreated plots increased during the growing season, as shown by an interaction between treatment and sampling date in the statistical analysis ( $F_{3, 899} = 12.39$ ,  $P < 0.001$ ).

Grain yield was significantly reduced ( $F_{1, 24} = 8.30$ ,  $P = 0.008$ ) as a consequence of weevil feeding (Table 3.1). The yield loss is ranged from 1.1% (XP1003) to 23% (Cypress). Planned F tests showed that the yields of “Bengal” and “Cypress” differed significantly between fipronil-treated and untreated plots, whereas yields of “Cocodrie” and “XP1003” did not differ significantly in treated and untreated plots. Panicle density was reduced by weevil feeding ( $F_{1, 28} = 29.04$ ,  $P < 0.001$ ). The reduction in panicle density was significant for varieties “Cocodrie”, “Cypress” and “XP1003”. Weight of 1000 grains was also reduced by weevil feeding (Table 3.1,  $F_{1, 26} = 11.66$ ,  $P = 0.002$ )

with the difference significant for “Bengal” and “Cypress”. Overall, the number of grains per panicle did not differ between treatments ( $F_{1, 25} = 2.45, P = 0.13$ ). However, the ANOVA showed a significant interaction between fipronil treatment and variety for this parameter ( $F_{3, 25} = 3.24, P = 0.04$ ). Planned F tests showed a significant reduction in number of grains per panicle for “Bengal” and “Cypress” but not for “Cocodrie” and “XP1003”.

### Discussion

The rice water weevil, *L. oryzaophilus*, has long been an important pest of rice in the U.S. and has recently emerged as a major pest of rice in Asia (Heinrichs & Quisenberry, 1999). Yield losses in excess of 20% have been reported in both Asia and the U.S. (Shang & Zhai, 1997; Stout et al., 2000). Development of sustainable management strategies for the rice water weevil will depend on an understanding of the mechanisms by which feeding of this pest reduces yields. Previous reports have implicated reductions in tillering as a major mechanism of yield loss (Grigarick, 1984; Hesler, et al. 2000). In the experiments reported here, we confirm this effect and, in addition, document for the first time effects on two other yield components, number of grains per panicle and grain weight. Thus, the effects of weevil feeding on rice development are more complex than previously documented. Moreover, we also show that the impact of weevil feeding differs among variety.

Injury and yield loss in rice infested by *L. oryzaophilus* was ultimately caused by the removal of root tissue by larvae. For instance, in 2001, reduction in mean root biomass across all four varieties averaged 54% at 64 days after flooding. The results from two years of experiments with four varieties show a consistent pattern of effects of this root

pruning on rice growth and development. In the vegetative stage, removal of root tissue led to reductions in tillering and in total shoot biomass. In the 2001 experiment, reductions across all four varieties in mean shoot biomass and tiller number were 35% and 36% at 64 days after flooding. Although previous studies have not documented reductions in shoot biomass following weevil feeding in rice, reductions in plant height, another measure of vegetative growth, have been noted (N'Guessan et al., 1994). At the reproductive stage, panicle density, grain weight and grains per panicle were reduced, Panicle density and grain weight were reduced by 12% and 2% respectively.

Injury by *L. oryophilus* larvae is a chronic process. This was shown by the statistical interactions between sampling date and tiller number, shoot biomass, and root biomass. Infestations of weevil larvae on roots began almost immediately after flooding, when rice plants in these experiments had just begun to tiller. Effects on rice growth, however, were not manifested until much later, at the internode elongation stage of rice growth. In the case of tillering, for example, no significant differences were observed in emergence of tillers, but a greater proportion of tillers senesced in infested plots than in fipronil-treated plots.

As has been previously documented, one of the major mechanisms by which rice water weevil reduce yields in this study was a reduction in tillering in weevil-infested plots. Tillering has been shown to significantly influence future production of panicles in rice (Miller et al., 1991; Wu et al., 1998); panicle density, in turn, is highly correlated with grain yield (Counce & Wells, 1990; Gravois & Helms, 1992). For “Cocodrie”, the increased yield loss from 11.7% in 2001 to 20.5% in 2000 was positively correlated with the greater reduction in panicle densities, from 21.6% in 2001 to 53.1% in 2000.



However, tiller number was not always correlated with panicle density. For example, there was a large difference between treatments in tiller number for “Bengal” in 2001, but the number of panicles for this variety was not significantly different between treatments. Conversely, the difference between treatments in tiller number for “Cypress” was small, but the difference in panicle density was large. One reason for these discrepancies may be related to senescence of tillers after the internode growth stage. Tiller senescence may be variable across variety and treatments. For “Cypress”, the tiller senescence rate may have been higher in fipronil-treated plots than in untreated-plots, whereas the rate may have been lower in “Bengal”.

The mechanism by which pruning of roots leads to reductions in tillering is not known. Tillering ability is influenced by many environmental factors; in particular, tillering is closely associated with the nutritional condition of the mother culm, because a tiller receives carbohydrate and nutrients from the mother culm during its early growth (Murayama 1964). Damage to root systems may limit nutrient uptake by rice plants and thereby reduce tillering. Data from fertilization experiments, however, suggest that reductions in nutrient uptake are not responsible for reductions in tillering in weevil-infested rice. In these experiments, increasing rates of fertilization of rice in the presence of weevil infestations did not compensate for weevil damage (Stout, unpublished data). Alternatively, damage to roots may reduce tillering more directly, perhaps by altering levels of hormones (e.g., cytokinin) in rice plants. This hypothesis will be tested in future experiments.

Reduction in tillering and resultant reduction in panicle density was not, however, the only mechanism whereby weevil feeding reduced yields. Yield losses also resulted from

reductions in the number of grains per panicle and in average grain weights. Effects on grain number and weight may have been caused by the reductions in shoot biomass observed in weevil-infested plants in this study. Reduction in shoot biomass probably resulted in a reduction in total leaf area, total plant photosynthesis, and stem carbohydrate levels. These factors play an important role in the grain-filling process, since the majority of photosynthates and nutrients used for grain-filling come from the upper leaves and from reserves in the stem of rice plants (Yoshida, 1981; Dunand, 1999; Sheehy, 2000). Thus, decreases in grain numbers and grain weight may be due to less photosynthesis or stored resources in weevil-damaged plants.

These experiments clearly show that there are differences among varieties in responses to rice water weevil injury, although, because the experiments involved only four varieties, three of which were tested in only one year, the data must be interpreted with caution. The cultivars “Cocodrie” and “XP1003” suffered lower yield losses than the other cultivars. This result was similar to results found by Stout et al. (2001), who reported that “Cocodrie” was more tolerant of weevil injury than “Bengal” and “Cypress”. In addition, weevil feeding affected the yield components of different cultivars differently, although overall trends were consistent among varieties. Reductions in number of grains per panicle and grain weight were more important contributors to yield loss in “Bengal” and “Cypress” than in “XP1003” and “Cocodrie”. Decreases in panicle densities appeared to be more important in “Cypress”, “XP1003” and “Cocodrie” than in “Bengal”. Further studies of these differences in responses are needed to determine how they contribute to differences in tolerance of weevil injury among varieties.

In addition to differences among varieties, there were some inconsistencies in the effects of weevil feeding on yield components for the variety “Cocodrie” in 2000 and 2001 (Table 3.1). Yield losses were greater, and effects on yield components more severe, in 2000 than in 2001. There are several possible explanations for these differences between years. Populations of *L. oryzaophilus* larvae in 2000 were higher than in 2001. From 20 to 30 days after flooding, when rice is very susceptible to weevil damage (Stout et al., 2002), the number of larvae per core was about 10-20% higher in 2000 than in 2001. In addition, fipronil excluded weevils from fipronil-treated plots more effectively throughout the season in 2000 than in 2001 (Fig. 3.1). As a result of these two factors, the difference in larval numbers between treated and untreated plots was much greater in 2000 than 2001. In addition, planting density was reduced from 112 kg ha<sup>-1</sup> in 2000 to 40 kg ha<sup>-1</sup> in 2001 to facilitate sampling of plants. Plant density is an important factor affecting tillering (Counce et al., 1992) because of the influence of plant density on competition for light and nutrition. Thus, yield losses in 2001 may have been lower because plants in this year were subject to less competition. Since tolerance characteristics are influenced by environment (Strauss & Agrawal, 1999), such differences between years are expected.

In conclusion, feeding by rice water weevil has chronic effects on multiple vegetative and reproductive characters in rice. Reduction in tillering is important, but other yield components (number of grains per panicle and grain weight) are affected as well. The effect of weevil feeding on vegetative is chronic. Moreover, effects variable among varieties. This study constitutes a first step toward a deeper understanding of the physiological effects of weevil feeding on rice physiology.

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## CHAPTER 4

### DENSITY-YIELD RELATIONSHIPS FOR RICE WATER WEEVIL ON RICE FOR DIFFERENT VARIETIES AND UNDER DIFFERENT WATER MANAGEMENT REGIMES

#### Introduction

The rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, is the most destructive insect pest of rice in the United States, and has recently assumed global importance due to its introduction into rice-producing regions of Asia (Heinrichs and Quisenberry, 1999). Both adults and larvae of *L. oryzophilus* feed on rice, but it is generally the larval stages that cause yield losses (Way, 1990). Adults feed on young rice leaves in flooded or unflooded rice fields, leaving feeding scars parallel to the venation of leaves. Oviposition begins when fields are flooded (Everett and Trahan, 1967). Larvae may feed on or in the leaf sheath for a short time after eclosion, but eventually move down to feed on or in roots, progressing through four instars in approximately 21-27 days under field conditions (Cave et al. 1983; Smith 1983; Zou and Stout, unpublished data). Root feeding by larvae results in reductions in vegetative growth, tillering, grain number, and grain weight (Zou et al., 2004). Yield losses caused by *L. oryzophilus* in Louisiana, where this insect is a particularly severe pest, typically exceed 10%, and can reach 25% or more under severe infestations (Smith 1983; Stout et al. 2000).

Louisiana rice producers currently rely almost exclusively on insecticides to manage the rice water weevil. The carbamate insecticide carbofuran (Furadan<sup>®</sup>, FMC) was used to manage the rice water weevil until the late 1990's, when the registration for use of this insecticide in rice was cancelled. Following this regulatory action, four insecticides were registered for use against the rice water weevil: fipronil (Icon<sup>®</sup> 6.2 FS, Bayer), lambda-

cyhalothrin (Karate Z<sup>®</sup>, Syngenta), zeta-cypermethrin (Fury<sup>®</sup>, FMC) and diflubenzuron (Dimilin<sup>®</sup>, Crompton). Proper use of these newly-registered insecticides represents a considerable challenge for rice producers. Carbofuran was a larvacide applied to flooded soils when densities of larvae exceeded a threshold density. In contrast, fipronil is a larvacide used as a prophylactic seed treatment, lambda-cyhalothrin and zeta-cypermethrin target adults, and diflubenzuron targets eggs through adult consumption. All three of the latter insecticides are applied to rice foliage shortly after fields are flooded. However, no empirically-derived adult thresholds have been developed in Louisiana to govern the timing of post-flood applications of lambda-cyhalothrin, zeta-cypermethrin and diflubenzuron.

Concerns about the toxicity of the newly-registered insecticides to non-target organisms and about the long-term sustainability of heavy reliance on insecticides have prompted investigations of alternative strategies for management of the rice water weevil (Stout et al., 2001). Several studies have demonstrated that short delays in flooding can reduce yield losses from the rice water weevil (Rice et al., 1999; Stout et al., 2001). This strategy is effective because oviposition does not occur until fields are flooded (Everett and Trahan, 1967; Stout et al., 2002a) and older plants are more tolerant of larval feeding than younger plants (Wu and Wilson 1997; Stout et al. 2002b). In addition, commercial rice varieties vary in their tolerance of feeding by weevil larvae, and use of more tolerant varieties may reduce losses from this insect (Stout et al., 2001). Theoretically, these alternative management strategies are compatible with the use of insecticides.

Quantifying the relationship between rice yield and density of *L. oryzaophilus* larvae (the damaging stage of this pest) is critical to the development of a management program



that integrates multiple strategies (Pedigo et al., 1986). A quantitative understanding of this relationship, together with an understanding of the relationship between adult density and larval density, will allow the development of thresholds for post-flood applications of insecticides. However, delays in flooding or use of tolerant varieties may alter the relationship between density of weevil larvae and yield loss. Thus, density-yield studies conducted with different varieties and under different flooding regimes will be needed in order to develop an integrated management program.

Previous studies of the relationship between rice water weevil densities and rice yields are not adequate for the development of a modern integrated management program against this insect in Louisiana. These prior studies were of a preliminary nature, used obsolete or older varieties, or were conducted in areas where rice production practices differ significantly from practices used in Louisiana (Tugwell and Stephen, 1981; Tsuzuki et al., 1983; Barbour et al., 1996). Moreover, none of these studies investigated the effects of different varieties or flooding regimes on density-yield relationships. The objective of this study was to characterize the relationship between rice yields and densities of *L. oryzaophilus* larvae using two varieties under two different flooding regimes. The varieties used, 'Cocodrie' and 'Bengal', had previously been shown to differ in their tolerance to the rice water weevil (Stout et al., 2001). On the basis of prior research (Rice et al., 1999; Stout et al., 2001, 2002a, 2002b), it was hypothesized that yield losses would be greater in early-flood plots than in delayed-flood plots, and greater in the susceptible cultivar 'Bengal' than in the more tolerant cultivar 'Cocodrie'. In addition, larval densities at early growth stages of rice were expected to correlate more strongly with yield losses than larval densities at later growth stages. The data obtained

provide a foundation for the establishment of a pest management program for rice water weevil that integrates use of insecticides, cultural practices, and plant resistance.

## **Material and Methods**

### **General Cultural Practices**

Experiments were conducted in 2001 and 2002 at the Louisiana State University AgCenter Rice Research Station (Crowley, Acadia Parish, LA). The soil type was a silt loam (fine, montmorillonitic, thermic typic albaqualf). For the 2001 experiment, rice ('Cocodrie') was drill-seeded on 17 April at a rate of 112 kg ha<sup>-1</sup>. Plots measured 1.5 m by 6 m and consisted of 7 rows of rice. Plots were irrigated on 18 April to encourage synchronous emergence. Permanent flood was applied on 11 May, 24 days after planting, to plots assigned to the "early flood" treatment; plants were at the 3-4 leaf (V3-V4) stage at this time (Counce et al., 2000). Plots assigned to the "delayed flood" treatment were flooded on 25 May, 38 days after planting, when plants were at the 6-7 leaf (V6-V7; mid-tillering) stage. In 2002, rice ('Cocodrie' and 'Bengal') was drill-seeded on 3 May at a rate of 100 kg ha<sup>-1</sup>; plot size was the same as 2001. Plots were irrigated on 9 May. Permanent flood was applied on 31 May (28 days after planting, plants at the early tillering stage) for early-flood plots and on 14 June (42 days after planting, plants at the late tillering stage) for delayed-flood plots. For both years, total nitrogen fertilization rate was 135 kg N/ ha with all N applied before flooding. Other agronomic practices used were typical of those used in southwest Louisiana. Rice was harvested on 23 August, 2001 and 6 September, 2002. Grain yield and moisture were determined simultaneously in the field by the harvester.

## **Experimental Design and Procedures**

The design of the experiment in 2001 was a split plot. The main plot factor was flooding regime (“early” or “delayed”); main plots were arranged according to a randomized complete block design with three replications. The sub-plot factor consisted of six levels of insect exclusion achieved using six rates of the insecticidal seed treatment fipronil (Icon<sup>®</sup>, Aventis Crop Science, Research Triangle Park, NC). Rice seed was treated at rates of 0.068, 0.023, 0.011, 0.0068, 0.0034, and 0 Kg AI (Active Ingredient)/ha.

The experiments in 2002 used two commercial varieties, “Cocodrie” and “Bengal”. These varieties are modern varieties that had been previously shown to differ in their tolerance to rice water weevil feeding (Stout et al., 2001). Two separate experiments were conducted with these two varieties, one in which plots were flooded at the 3-4 leaf stage (early tillering, “early flood”) and one in which flooding was delayed until plants were at 6-7 leaf stage (late tillering, “delayed flood”). Because timing of flood was investigated in separate experiments in 2002 it could not be included as a replicated factor in statistical analyses. Timing of flood was investigated in separated experiments in 2002 due to constraints on available land and labor, and because the effect of timing of flood had been established in the previous year’s experiment and in other experiments (Stout et al., 2001; 2002a). The experimental design for both early and delayed-flood experiments was a randomized complete block with 12 treatments and three replications. The 12 treatments were factorial combinations of two varieties and six fipronil treatments. Fipronil treatments were the same as in 2001. Grain yield and moisture were determined in the field by the harvester.

## **Sampling of Insects**

Densities of *L. oryzaophilus* larvae and pupae were determined by taking root/soil core samples from plots (Stout et al., 2000). The core sampler was a metal cylinder with a diameter of 9.2 cm and a depth of 7.6 cm attached to a metal handle. Core samples were taken from the interior 5 rows of plots. Larval densities were estimated by taking three core samples per plot. Cores were placed into 40 mesh screen sieve buckets and soil was washed from roots. Buckets were placed into basins with salt water, and larvae and pupae were counted as they floated to the water surface (Everett 1963). In 2001, sampling of larvae was conducted on three dates after permanent flood (daf) for both early and delayed-flood plots: 19 days (30 May), 30 days (10 June) and 40 days (20 June) for early-flood plots and 21 days (15 June), 32 days (26 June) and 40 days (4 July) for delayed-flood plots. In 2002, sampling of larvae was conducted on two dates after permanent flood: 20 days (20 June) and 32 days (2 July) for the early-flood experiment and 24 days (8 July) and 33 days (17 July) for the delayed-flood experiment.

## **Statistical Analyses**

Average densities of immature weevils in each plot on each sampling date were calculated by taking the mean of the three core samples. Average densities were used in all analyses. Immature weevils consisted primarily of larvae (> 96%), with only a few pupae. Grain yields were adjusted to 12% moisture before conducting analyses. A normalized yield for each plot was used for analyses, allowing generalization to other experiments and years. Normalized yield was obtained by dividing raw yields by the yield from the plot in the same block treated with the highest rate of fipronil, and

normalized yield loss was calculated by subtracting the normalized yield from 1 (1-(yield /yield with 0.068 Kg AI/ha fipronil )).

A mixed-model, split-plot repeated measures analysis was used to investigate the effect of flooding regime and insecticide on yield losses and insect densities in the 2001 experiment. This analysis was intended to evaluate the overall effect of flooding regime and insecticide treatment on yield loss and insect density. The analysis was conducted using PROC MIXED of SAS (Littell et al., 1996), with block (replicate) as a random effect, flooding regime and rate of fipronil as fixed effects, and sampling date as a repeated measure. Similar mixed model analyses with variety as a fixed effect were used to compare yield losses in the varieties ‘Bengal’ and ‘Cocodrie’ in the 2002 experiments. Two analyses were conducted, one for the “early flood” experiment and one for the “delayed flood” experiment. Analyses were conducted using PROC MIXED in SAS with normalized yield loss or density of immatures as the dependent factor, variety and rate of fipronil as fixed effects, block (replicate) as a random effect, and sampling time as a repeated measure. For all mixed model analyses, the Satterthwaite option in SAS was used to determine the correct degrees of freedom for estimates and tests of interest (Littell et al. 1996).

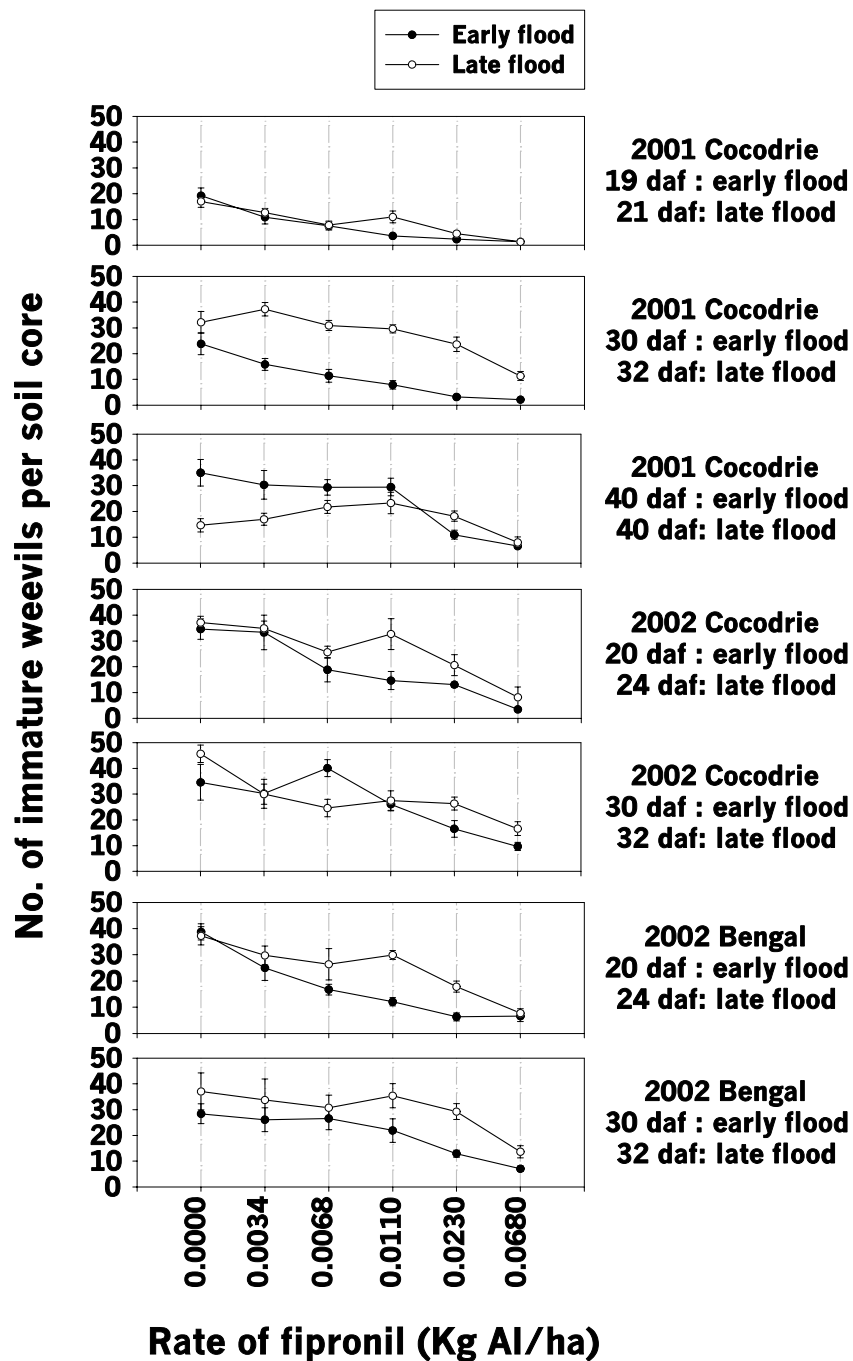
Relationships between densities of immatures and normalized yields were analyzed using regression. The dependent variable in all analyses was normalized yield; the independent variables were densities of immatures from individual sampling dates for simple regression and from all samplings dates for multiple regression. The best models were selected on the basis of multiple criteria: mean square error (MSE), coefficient of determination ( $r^2$ ) and coefficient of multiple determination ( $R^2$ ) for multiple regression,

and Mallow's Cp (Myers, 1990). Best-fit models are characterized by small MSE, large  $r^2$  or  $R^2$  values, and a Cp smaller than or close to the number of terms in the model, including intercept. Selections were made from linear, quadratic and cubic models until the best fit model was found. Values of MSE and Cp were obtained using PROC SQUARE in SAS. These two parameters were used to select the optimal subsets of models. Slopes and intercepts were estimated using PROC GLM statements in SAS (SAS Institute, 1991). Intercepts were restricted to one for models with untransformed dependent variables to suppress the inflation of  $r^2$  or  $R^2$  (Myers, 1990).

### Results

Densities of the immature stages of *L. oryzaophilus* (principally larvae) decreased as fipronil rate increased in 2001 ( $F_{5, 180} = 43.34$ ,  $P < 0.001$ , Fig. 4.1). Larval densities differed significantly with sampling date ( $F_{2, 180} = 80.61$ ,  $P < 0.001$ ), with densities on the first sampling date lower than on the 2<sup>nd</sup> and 3<sup>rd</sup> sampling dates. Larval densities in 2001 were also affected by flooding regime date ( $F_{1, 180} = 21.51$ ,  $P < 0.001$ ). Densities of immatures averaged across all sampling dates and rates of fipronil were lower in early-flood plots than delayed-flood plots (Table 4.1). The higher larval densities in delayed-flood plots may have resulted from degradation of fipronil activity over time (sampling of delayed-flood plots was delayed relative to sampling of early-flood plots). There were significant interactions for larval densities among flooding regime, insecticide treatment and sampling dates ( $F_{10, 180} = 2.77$ ,  $P = 0.003$ ).

The general patterns of immature densities in the 2002 experiments were similar to those in 2001 (Fig. 4.1), but average densities of rice water weevil larvae were higher. Densities again differed with sampling date ( $F_{1, 36} = 11.22$ ,  $P = 0.002$  in early-flood plots



**Figure 4.1** Densities of immature rice water weevils (number of larvae and pupae per core  $\pm$  S.E.) under early flood (filled circles) and delayed-flood regime (open circles), in 2001 and 2002. Points along the X axis represent rates of fipronil in ascending order. For the 2001 experiment, densities of immatures were estimated by taking core samples at 19, 30 and 40 days after flooding (daf) for early-flood plots and 21, 32 and 40 daf for delayed-flood plots. For the 2002 experiment, core samples were taken from plots of Bengal and Cocodrie rice at 20 and 32 daf for early-flood plots and 24 and 33 daf for delayed-flood plots.

**Table 4.1** Effects of flooding regime (2001 experiment) and variety (2002) on standardized yield losses and larval densities. Yield losses and larval densities shown are averaged over all insecticide treatments.

Yield, Yield loss and Larval density	2001 Flooding regime		2002 Varieties in early-flood plots		2002 Varieties in delayed-flood plots	
	Early	Late	Bengal	Cocodrie	Bengal	Cocodrie
	Yield (Kg/ha) <sup>a</sup>	5843.1 ± 266.1	7419.7 ± 107.4	4910.3 ± 239.9	5427.7 ± 283.5	6089.8 ± 161.7
Yield loss (%)	22.5 ± 3.4 <sup>d</sup>	8.3 ± 1.6	23.2 ± 3.8	23.8 ± 4.0	19.1 ± 2.2 <sup>d</sup>	10.1 ± 1.5
Larval density (larvae per core)	13.9 ± 1.4 <sup>d</sup>	17.9 ± 1.0	19.0 ± 2.1 <sup>b</sup>	22.9 ± 2.3	27.4 ± 2.0	27.5 ± 2.1

<sup>b</sup>  $p < 0.05$ ; <sup>c</sup>  $p < 0.01$ ; <sup>d</sup>  $p < 0.001$ ; Planned-comparisons of yield losses and larval densities were made between flooding regimes (2001) and varieties in early and delayed-flood regimes (2002). Superscript letters b, c, d show the significant levels for treatment effects. Superscript a means raw yields were not statistically analyzed.



and  $F_{1, 72} = 4.43$ ,  $P = 0.039$  in delayed-flood plots). Larval density was significantly higher in “Cocodrie” plots compared to “Bengal” plots in the early-flood experiment ( $F_{1, 36} = 5.75$ ,  $P = 0.022$ , Table 4.1). Density did not differ between the two varieties in the delayed-flood experiment ( $F_{1, 72} = 0.01$ ,  $P = 0.956$ , Table 4.1). The only significant interaction was between insecticide treatment and sampling time in early-flood plots ( $F_{5, 36} = 4.82$ ,  $P = 0.002$ ).

The effect of flooding regime on yield loss in 2001 was significant ( $F_{1, 204} = 183.82$ ,  $P < 0.001$ , Table 4.1). Normalized yield losses in early-flood plots in 2001 were significantly higher than in delayed-flood plots (Table 4.1) despite the fact that insect densities in delayed-flood plots were, on average, higher. Thus, delaying flood by a short period of time (2 weeks) reduced normalized yield loss by 14.2% (22.5%-8.3%). There was an significant interaction for yield loss between flooding regime and insecticide treatment ( $F_{5, 204} = 47.59$ ,  $P < 0.001$ ).

A similar pattern was found in 2002 (Table 4.1). Normalized yield losses were reduced by 4.1% (23.2%-19.1%) for Bengal and 13.7% (23.8%-10.1%) for Cocodrie in delayed-flood plots compared to early-flood plots (Table 4.1). Yield losses from plots of Cocodrie and Bengal differed under the delayed-flood regime ( $F_{1, 81} = 107.37$ ,  $P < 0.001$ ). The average yield loss for Bengal in the delayed-flood plots was 19.1%, higher than the 10.1% yield loss observed in plots of Cocodrie in the same experiment (Table 4.1). There was no significant difference in yield losses suffered by the two varieties in early-flood plots ( $F_{1, 3} = 0.23$ ,  $P = 0.661$ , Table 3.1). There was an interaction between variety and insecticide treatment in the delayed-flood experiment ( $F_{5, 81} = 5.26$ ,  $P = 0.001$ ).

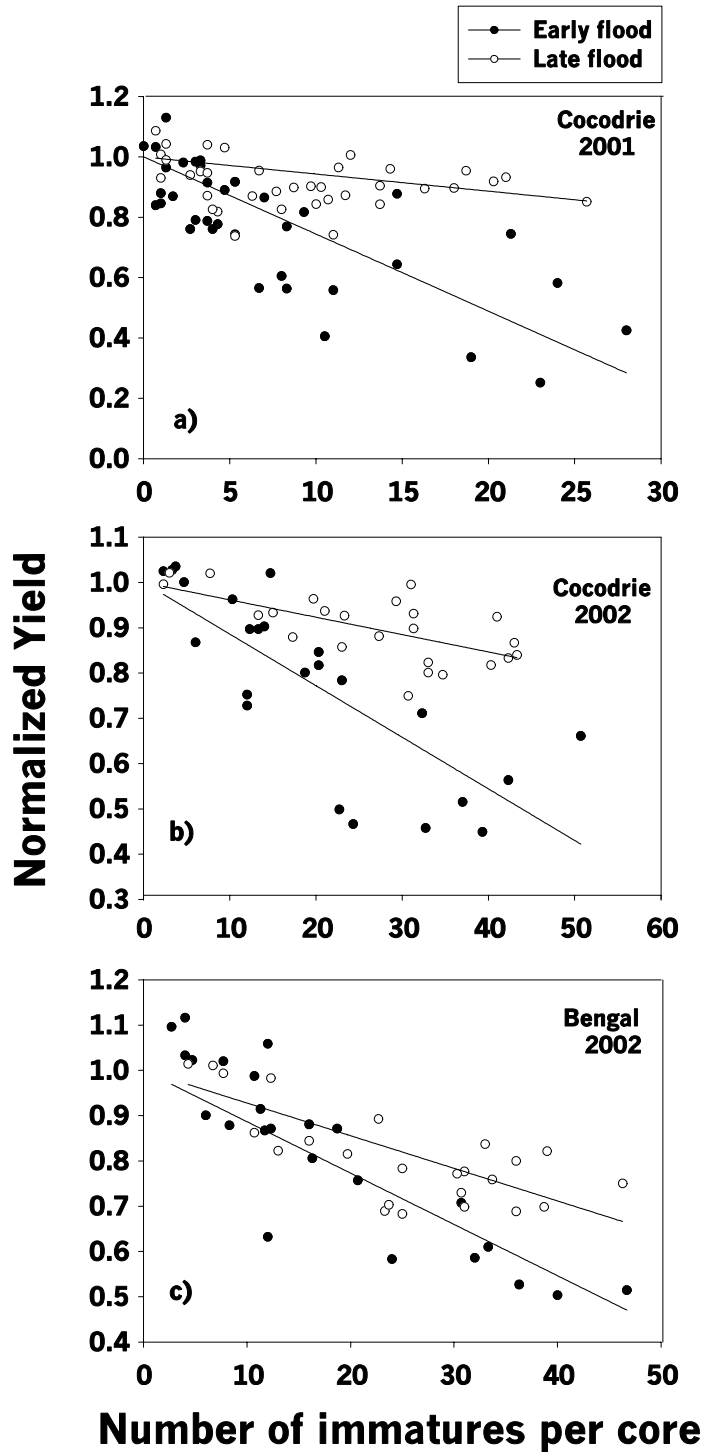
Plots in both years experienced a range of infestation pressures, making regression analyses appropriate. Separate regression models were developed for each core sampling date in each experiment to describe relationships between densities of immature weevils and yields. In general, these relationships were best described by linear models (Table 4.2). Only linear models are presented to facilitate comparison between models and facilitate application to IPM decision making. The slopes provide estimates of the amount of yield loss caused by each rice water weevil larva. These estimates differed substantially in early-flood and delayed-flood plots. Slopes of relationships between larval densities (from earliest core sampling dates) and yield were less negative in delayed-flood plots than in early-flood plots (Fig. 4.2). For early-flood plots, yield loss per larva using larval densities from first core sampling dates were 4.12% (Cocodrie; 2001), 1.19% (Cocodrie; 2002), and 1.31% (Bengal; 2002). For delayed-flood plots, estimates ranged from 1.53% (Cocodrie; 2001) to 0.38% (Cocodrie; 2002) and 0.64% (Bengal; 2002).

Regression models also differed depending on the core sampling date used to construct the models. Correlations of yields and densities of immature weevils from the third core sampling dates in both early and delayed-flood plots in 2001 were much weaker than correlations of yields and weevil densities from the first and second sampling dates (Table 4.2). Using the sum of immature densities over the three core sampling dates as an independent variable marginally improved some models, but did not improve or weakened most models. Multiple regression analyses revealed that, for four out of six cases, only densities from the first sampling date were significantly related to yield loss and that densities of weevils from the third core sampling were statistically

**Table 4.2** Parameters and statistics for best-fit regression models developed to describe relationships between standardized yields and larval densities of *L. oryzaophilus* for two varieties of rice grown under different water management regimes in 2001 and 2002. Separate models were developed using larval densities from each of three (2001) and of two core sampling dates (2002).

Variety year	Flooding regime	Sampling date	Equation	Model parameters			
				P	MSE	r <sup>2</sup>	Cp
Cocodrie 2001	Early	19D	Y=1.0-0.0257X	< 0.0001	0.0163	0.6104	6.16*
		30D	Y=1.0-0.0189X	< 0.0001	0.0194	0.5374	9.91*
		40D	Y=1.0-0.0095X	< 0.0001	0.0242	0.4212	5.15*
		Total1	Y=1.0-0.0120X	< 0.0001	0.0122	0.7086	4.20*
		Total	Y=1.0-0.0057X	< 0.0001	0.0134	0.6810	0.14
	Late	19D	Y=1.0-0.0074X	< 0.0001	0.0088	0.0707	4.87*
		30D	Y=1.0-0.0031X	< 0.0001	0.0076	0.1901	0.97
		40D	Y=1.0-0.0045X	< 0.0001	0.0088	0.0651	0.96
		Total1	Y=1.0-0.0120X	< 0.0001	0.0077	0.1808	1.25
		Total	Y=1.0-0.0057X	< 0.0001	0.0076	0.1961	2.25
Cocodrie 2002	Early	20D	Y=1.0-0.0119X	< 0.0001	0.0145	0.6326	5.19*
		32D	Y=1.0-0.0089X	< 0.0001	0.0274	0.3069	2.68
		Total1	Y=1.0-0.0055X	< 0.0001	0.0155	0.6078	0.63
	Late	20D	Y=1.0-0.0038X	< 0.0001	0.0038	0.3753	0.18
		32D	Y=1.0-0.0037X	< 0.0001	0.0032	0.4697	1.41
		Total1	Y=1.0-0.0019X	< 0.0001	0.0028	0.5291	0.31
Bengal 2002	Early	20D	Y=1.0-0.0131X	< 0.0001	0.0080	0.7769	1.38
		32D	Y=1.0-0.0115X	< 0.0001	0.0191	0.4657	2.56
		Total1	Y=1.0-0.0065X	< 0.0001	0.0088	0.7552	0.67
	Late	20D	Y=1.0-0.0075X	< 0.0001	0.0059	0.4980	9.08*
		32D	Y=1.0-0.0064X	< 0.0001	0.0052	0.5552	7.71*
		Total1	Y=1.0-0.0036X	< 0.0001	0.0026	0.7748	10.62*

19, 20, 30D etc. refer to number of days after flooding; X = larval density; Y = standardized yield; Cp = Mallow's Cp; MSE = mean square error; r<sup>2</sup> = coefficient of determination; Total1=sum of average densities from first two core samples. Total = sum of all three core samples (for 2001 only). \* denotes that Cp value is high, a bias may occur.



**Figure 4.2** Relationships between densities of rice water weevil immatures and standardized yields of two rice varieties, “Cocodrie” and “Bengal”, under early-flood (filled circles ) and delayed-flood regimes (open circles) in 2001 and 2002. The immature densities are from the earliest samples (19 daf for early-flood plots and 21 daf for delayed-flood plots in 2001; 20 daf for early-flood plots and 24 daf for delayed-flood plots in 2002). Best fit regression models are shown.

**Table 4.3** Parameters and statistics for best-fit models (multiple regression) developed to describe relationships between standardized yields and larval densities of *L. oryzaophilus* for two varieties of rice grown under different water management regimes in 2001 and 2002.

Variety Year	Flooding regime	Equation	Model parameters			
			P	MSE	R <sup>2</sup>	Cp
Cocodrie 2001	Early	Y=1.0-0.0151SD1-0.0097SD2	< 0.0001	0.0121	0.7189	3.09
	Late	Y=1.0-0.0074SD1	< 0.0001	0.0088	0.0707	4.19*
Cocodrie 2002	Early	Y=1.0-0.0119SD1	< 0.0001	0.0145	0.6326	5.19*
	Late	Y=1.0-0.0038SD1	< 0.0001	0.0038	0.3753	7.16*
Bengal 2002	Early	Y=1.0-0.0013SD1	< 0.0001	0.0080	0.7769	2.48
	Late	Y=1.0-0.0036SD1-0.0036SD2	< 0.0001	0.0028	0.7749	2.69

SD1, SD2, SD3= larval density at first sampling date, second sampling date and the third sampling date. Other variables are the same as variables in table 1.1. \* denotes that Cp value is high, a bias may occur.

unrelated to yield loss (Table 4.3). Furthermore, the slopes of the relationships between yields and insect densities on the first sampling dates were always more negative than the slopes of the relationships between yields and densities on the second or third sampling dates (Table 4.2).

## Discussion

Relationships between rice yields and densities of the rice water weevil, *L. oryzaophilus*, were quantified under two flooding regimes and for two varieties. Density-yield relationships were substantially affected by both flooding regime and variety. These data are critical for developing management programs in which multiple control strategies, including insecticides, are used effectively. Our results showed that short delays in flooding reduced yield losses from the rice water weevil in both 2001 and 2002. Similar reductions in yield losses were reported in earlier studies from Louisiana (Rice et al., 1999; Stout et al., 2001). Stout et al. (2001) found that the average yield loss across eight varieties was 22.5% in early-flood plots but only 10.8% in delayed-flood plots.

Stout et al. (2002b) provided evidence that the tolerance of rice to feeding by rice water weevil larvae increases with plant age. The results of these experiments provide additional support for this hypothesis. Regression analyses showed that larval densities during earlier stages of rice growth were more strongly correlated with yield losses than larval densities later in the growing season. In addition, slopes of the relationships between larval densities and yields were more strongly negative for early sampling dates than for later sampling dates. These facts indicate that larval infestation during the earlier growth stages of rice contribute more to yield loss than does infestation during later

growth stages. Thus, delayed flooding allows plants to reach a more tolerant growth stage before infestation.

Yield losses in the 2002 experiment were greater for 'Bengal' than for 'Cocodrie'. However, this difference between varieties was found only in delayed-flood plots; there were no differences in yield losses between varieties for early-flood plots. These data are consistent with data reported by Stout et al. (Stout et al., 2001) in which it was also found that Cocodrie was more tolerant than Bengal, and that differences in tolerance among varieties were more evident in delayed-flood plots. Early flooding, which results in heavy infestations of young, vulnerable plants, may mask differences in tolerances among varieties.

The regression models developed in this study were used to obtain estimates of damage caused by each weevil (injury units per insect \* damage per unit injury), a key parameter for the calculation of economic injury levels (Pedigo et al., 1986). These estimates were obtained by using regression equations to calculate yield losses caused by a single larva. For example, yield loss per larva using larval densities from first core sampling dates are 4.12% and 1.53% for Cocodrie in 2001 and 2002 respectively. These estimates appear to be similar to those found by previous researchers (Grigarick, 1984; Way et al., 1991), although direct comparisons are impossible because of differences in methodologies, flooding regimes, varieties and the failure of previous studies to account for the effect of plant age on density-yield relationships. These approximations probably overestimate yield losses caused by each larva because larval density at a single core sampling date does not represent the entire total seasonal population of weevils in a rice field. Nonetheless, estimates of damage per weevil were greater in early-flood plots than

delayed-flood plots, and were higher for the less tolerant variety (Bengal) than for Cocodrie.

Effective use of newly-registered adulticidal and ovicidal insecticides in rice against the rice water weevil requires the development of economic thresholds based on adult densities. Previous attempts to directly establish relationships between adult densities and yield losses have failed because of difficulties in manipulating densities of adults in the field (Stout, unpublished data). The estimates of yield loss per larva obtained in the current study can be used to develop adult thresholds if relationships between adult density and larval density can be empirically established or estimated based on female fecundity. Efforts to quantify this relationship using various methods for monitoring adults are currently in progress.

Integration of control tactics is essential to achieve the IPM objectives of maintaining fluctuating pest populations below economic injury levels (EILs) and attenuating the risks of sole reliance on insecticides (Kogan, 1988). A more rational pest management program for the rice water weevil can be achieved by integrating the use of insecticides, delayed flooding, and plant resistance. Use of insecticides against the rice water weevil in rice can be reduced if levels of plant tolerance are sufficient to compensate (increased EILs) for increased populations of weevils that may result from reduced use of insecticides. Increases in crop tolerance can be achieved by using tolerant genotypes, and, operationally, by growing crops in environments that favor the expression of tolerance (Kennedy et al., 1987; Strauss and Agrawal, 1999). In rice, the use of cultural practices (such as delayed flooding) that delay infestations of weevil larvae until rice is older effectively increases the tolerance of rice to weevils. Moreover, delaying flood may



create an environment more suitable for expression of plant resistance. While this study provides a basis for integrating multiple tactics against the rice water weevil in rice, further work is needed to develop adult thresholds to govern effective and judicious use of insecticides in this system. In addition, further work is needed to develop rice with high levels of tolerance to the rice water weevil.

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## CHAPTER 5

### DEGREE DAY MODELS FOR EMERGENCE AND DEVELOPMENT OF THE RICE WATER WEEVIL IN SOUTHWEST LOUISIANA

#### Introduction

The rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, is the most destructive insect pest of rice in the United States, and has recently assumed global importance due to its introduction into rice-producing regions of Asia (Heinrichs and Quisenberry, 1999). Adult weevils overwinter in bunch grasses or in leaf litter in wooded areas and emerge in early spring (Morgan et al. 1984). Both adults and larvae of *L. oryzophilus* feed on rice, but it is generally the larval stage that causes yield losses (Way, 1990). Adults feed on young rice leaves in flooded or unflooded rice fields, leaving feeding scars parallel to the venation of leaves. Oviposition commences when fields are flooded (Everett and Trahan, 1967). Larvae may feed on or in leaves for a short period of time after eclosion, but eventually move down to the roots. Larvae feed on or in roots, and progress through four instars and a pupal stage on roots (Cave et al. 1983; Smith 1983). The rice water weevil is multivoltine in south Louisiana. Root pruning by larvae results in reductions in vegetative growth, tillering, grain number, and grain weight (Zou et al., 2004a). Yield losses caused by *L. oryzophilus* in Louisiana, where this insect is a particularly severe pest, typically exceed 10%, and can reach 25% or more under severe infestations (Smith 1983; Stout et al. 2000).

Development of an effective management program for *L. oryzophilus* will depend upon an understanding of the life history and population dynamics of this pest. For example, understanding rice water weevil oviposition behavior and, in particular, the dependence of oviposition on the presence of flood has led to investigations of delayed

flooding as a control tactic for the weevil (Everett and Trahan 1967, Rice et al. 1999, Stout et al. 2001, 2002a). Many behaviors and events in the life histories of insects can be predicted using temperature dependent (degree-day) models, because development of poikilotherms is directly related to temperature and time (Higley et al. 1986). Thus, development of appropriate degree-day models can facilitate the development of an IPM program for the rice water weevil in rice.

Two aspects of the life history of the rice water weevil that remain poorly understood are the emergence of adults from overwintering sites in spring and development of immature weevils on rice roots. Field observations of spring emergence range from late March to April, and emergence appears to be associated with the occurrence of several days with a mean daily temperature in excess of 15.6°C (Muegge et al. 1996). No quantitative models have been developed to predict the date of emergence in Louisiana. Similarly, little quantitative research has been conducted on the development of immature stages of weevils under field conditions. Raksarart and Tugwell (1975) measured the length of the egg stage at four temperatures in the laboratory. By periodically sampling rice plots in the field, the duration of the larval stage was estimated to range from 16 to 26 days on two rice genotypes (Cave et al. 1984). These data, however, are not adequate to quantify the development of a single generation of *L. oryzaophilus* under Louisiana field conditions. Duration of the egg stage estimated in the laboratory may not represent development under field conditions. Data were not recorded on the development of discrete instars of larvae and pupae and associated soil temperatures in the Cave et al. (1984) study. In addition, studies have not been conducted to compare development on recent commercial varieties that differ in their resistance to the rice water weevil.

The objectives of this study were to develop quantitative degree-day models to predict the emergence date of adult *L. oryzaophilus* from overwintering sites in the spring and to describe larval and pupal development of weevils on two varieties of rice under field conditions. The varieties used for the latter study, “Jefferson” and “Bengal”, had previously been shown to differ in their resistance to the rice water weevil (Stout et al. 2001). These models will provide basic information for the establishment of a pest management program for the rice water weevil that integrates the use of insecticides, cultural practices, and plant resistance.

## **Material and Methods**

### **Degree-day Model to Predict Spring Emergence**

The model was developed using light trap and weather data collected over 14 years at the Rice Research Station, Louisiana State University Agricultural Center, Crowley, Louisiana. The light trap was located between a wooded area and rice fields and was equipped with a 75-watt incandescent light bulb and a photo-switch that turned the light on at dusk. Weevils were trapped into 1 qt mason collection jars and were counted daily (Gifford and Trahan 1967). Although data were collected for more than 14 years, only data from years with complete records were used for model development. In addition, beginning in 2001, an ultraviolet (UV) light trap with a 4-watt bulb (model 2827D, BioQuip, Gardena, CA) was set up at the Rice Research Station. The light trap was connected to a bottle rotator (model 1512, John W. Hock Company, Gainesville, FL) that automatically rotated the bottles daily at dusk as determined by a programmable timer. Weevils were collected from early March through the end of the growing season in 2001, 2002 and 2003. These three years of data were used to verify the model developed from

the 14 year data set discussed above. Daily maximum and minimum temperatures were obtained from a weather station at the LSU AgCenter Rice Research Station.

Degree-days were calculated using the single sine method developed by Allen (1976). This method is more accurate than the traditional triangular method when minimum daily temperatures are below the development threshold (Pruess 1983). Previous laboratory and field studies estimated the low temperature threshold for flight muscle development of rice water weevils as 13.8°C (Matsui 1985) and 18°C (Morgan et al. 1984). The parameters of the model consisted of the low temperature threshold for degree-day accumulation, the cumulative number of weevils captured in light traps, and the start date for accumulation of degree-days. An iterative procedure was used to determine the combination of values for these parameters that minimized the difference between predicted and actual dates of emergence. Temperature thresholds were varied from 10°C to 21°C, cumulative catches of weevils were varied from 1 to 10, and start dates were varied from 1 to 60 days after January 1<sup>st</sup> for model development. A total of 12,000 combinations of parameters were used.

The iterative procedure was implemented using C programming language (Compiled by Microsoft Visual C++ 6.0). For each combination of parameters, the number of degree-days required to reach the emergence event was calculated for each year. A mean value was obtained by averaging the degree-days required to reach the emergence event over 14 years. The predicted date of event occurrence for each year was determined using this mean degree-day value. The predicted date of event occurrence was then compared with actual date of event occurrence. The root mean square error (RMSE) of the difference (in days) between actual date of event occurrence and predicted date of

event occurrence was calculated. The RMSE (RMSE = square root ((sum(predicted date-actual date)<sup>2</sup>)/14)) was used to measure how well the model predicted the date of event occurrence for each combination of parameters. The smaller the RMSE, the better the fit of the model. The combination of parameters having the smallest RMSE was chosen as the best-fit combination of parameters.

For model verification, the best-fit combination of parameters was used to calculate the predicted emergence date for 2001, 2002 and 2003. Predicted dates were compared with actual dates.

### **Larval Development Study**

Experiments were conducted in 2003 at the Louisiana State University Rice Research Station, Crowley, Acadia Parish, LA. The soil type was a silt loam (fine, montmorillonitic, thermic typic albaqualf). Three separate experiments were conducted throughout the growing season in an attempt to obtain adequate replication under a range of soil temperatures. In all three experiments, the intended experimental design was a randomized complete block with two commercial varieties, “Jefferson” and “Bengal”, and four replications. However, in the first experiment, seeds of “Jefferson” failed to germinate, leaving only 4 plots of “Bengal”. “Jefferson” and “Bengal” are recently-introduced varieties that had been previously shown to differ in their resistance and tolerance to the rice water weevil (Stout et al. 2001). Plots measured 1.2 meters by 6 meters and consisted of 7 rows of rice. A seeding rate of 90 kg ha<sup>-1</sup> was used for all experiments. For the first experiment, rice was drill-seeded on 26 March and plots were irrigated on 1 April to encourage synchronous emergence. Permanent flood was applied on 1 May. For the second experiment, rice was drill-seeded on 8 May, irrigated on 14



May and flooded on 5 Jun. For the third experiment, rice was drill-seeded on 27 May, irrigated on 29 May and flooded on 3 July. Total nitrogen fertilization rate was 68 kg N ha<sup>-1</sup> for all three experiments with all nitrogen applied before flooding. Other agronomic practices used were typical of those used in southwest Louisiana.

Rice plots were left untreated for one week after permanent flooding to allow mating and oviposition by natural infestations of weevils. Following this one week oviposition period, Karate<sup>®</sup> (lambda-cyhalothrin, Syngenta) was applied three times in the subsequent one week period at a rate of 0.057 Kg AI/ha (active ingredient per hectare) to eliminate adults and prevent subsequent oviposition. Dates of lambda-cyhalothrin applications were as follows: May 8, 12 and 16 for the first experiment, June 12, 17 and 20 for the second experiment, and July 11, 14 and 16 for the third experiment. Larvae and pupae of *L. oryzaophilus* were collected at several time points after the cessation of lambda-cyhalothrin applications by taking soil core samples from plots. The number of larvae found in each sample ranged from 10-30/plot. Plants were placed into 40 mesh screen sieve buckets and soil was washed from roots. Buckets were placed into basins with water, and larvae and pupae were collected as they floated to the water surface (Stout et al., 2000). A waterproof temperature logger (HOBO<sup>®</sup>, Onset Computer Corporation, Bourne, MA) was placed under the soil before the beginning of experiments to record the soil temperature once every 10 minutes.

Head capsules of collected larvae were measured under a microscope equipped with a eyepiece micrometer (MA524, 1mm divided into 100 units-minimum unit of 0.01mm, Meiji Techno. America, San Jose, CA). Larvae were categorized into instars using the classification method of Cave and Smith (1983) in which the head-capsule widths of each

instar were: 1<sup>st</sup>, 0.14-0.19 mm; 2<sup>nd</sup>, 0.19-0.27 mm; 3<sup>rd</sup>, 0.27-0.39 mm and 4<sup>th</sup>, 0.39-0.60 mm.

In the third experiment, pupal development was also monitored. Pupae were placed into three categories: pre-pupa, pupa and post-pupa. The pre-pupa was characterized by the presence of a 4<sup>th</sup> instar larva inside the cocoon (Chapman 1998). The pupal stage is the stage when the features of the adult become recognizable in the pupa (Chapman 1998). The post-pupa was defined in this study as the stage shortly before emergence of adults, characterized by a nearly fully developed adult of light yellow color inside the cocoon. The end point of pupal development for the purpose of model development was defined as the time when 50% of the weevils reached the post-pupal stage.

Data on the width of head-capsules were analyzed using a repeated-measures mixed model in SAS (Littell et al., 1996). This model was suitable for this set of data because data were collected from each experimental unit (plot) several times over the course of the growing season. The model considers block (replicate) as a random effect, and variety, sampling time and interaction terms as fixed effects. All data analyses were conducted using PROC MIXED of SAS (Littell et al., 1996).

The starting point for calculating degree-days was set as noon on the third day after flooding and the end point was defined as the day when 50% of the weevils reached the pupal stage. Oviposition was assumed to have been evenly distributed throughout the first week and oviposition was assumed to have ceased once lambda-cyhalothrin applications were made. Therefore, noon of the third day after flooding approximates the time at which half of the eggs were deposited. Under the assumption of no temperature change in each 10 minute period, a degree-day value for each day was calculated by

summing the effective heat units (recorded temperature – low temperature threshold) for all 10 minutes intervals then dividing by 144 (number of 10 minutes intervals per day).

The low temperature threshold for *L. oryzoophilus* development was estimated using thresholds of closely related species. Developed thresholds for other members of the family Curculionidae range from 7.0°C to 13.3°C (Guppy and Mukerji 1974, Simonet and Davenport 1981, Cacka 1982, Woodson and Eldelson 1988, Mazzei et al., 1999). The two most closely related species for which threshold data are available are in the genus *Listronotus* (Marvaldi et al. 2002). The temperature threshold for immature development in this genus was 7.0°C (Simonet and Davenport 1981) and 13.3°C for *L. oregonensis* and *L. texanus*, respectively (Woodson and Eldelson 1988). These two species are, like *L. oryzoophilus*, root feeding weevils. In Crowley, Louisiana, the minimum soil temperature after the end of January was always above 10°C (McClain and Sonnier 2001). Using the above information, 10°C was chosen as a reasonable low temperature threshold for *L. oryzoophilus*.

## **Results**

### **Predicting Emergence Date**

The smallest RMSE of 5.01 days was found using the following parameters: starting date, Feb 2 (day of year 33); temperature threshold, 15.6°C; cumulative catches of weevils, 6. Using these parameters, emergence of weevils occurred after 139.2 degree-days (°C × Day) were accumulated. The predicted dates of emergence using this model were April 4, 20 and 18 for 2001, 2002 and 2003 respectively. The actual dates of emergence (6 cumulative weevils in traps) in these years were April 8, 13 and 17 for

2001, 2002 and 2003 respectively. Prediction errors were 4, 7 and 1 days respectively for these three years (Fig. 5.1).

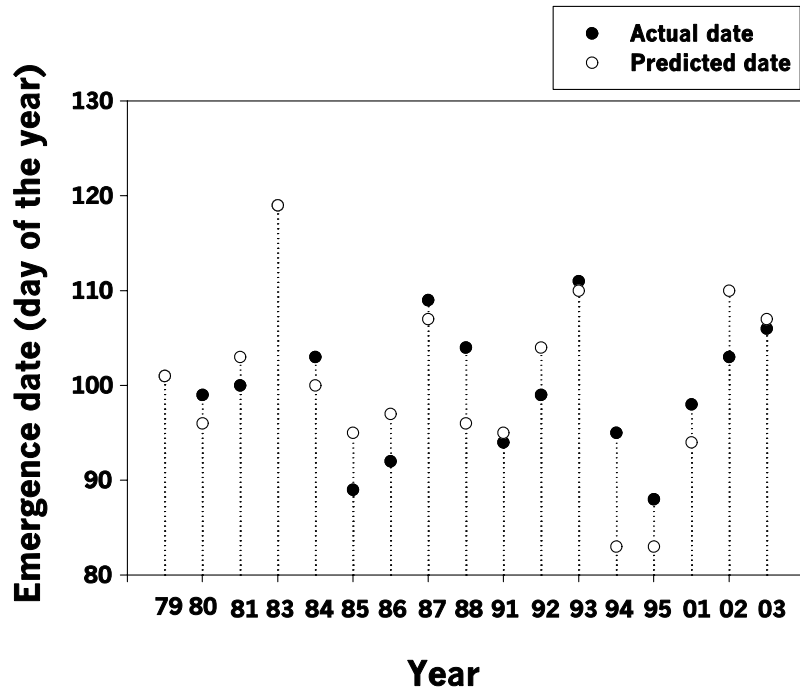
### **Larval Development Study**

The mean daily soil temperature increased from the first experiment (May) to the third experiment (August) with a maximum difference in mean temperature between these months of 7°C (Fig. 5.2). Soil temperatures were relatively stable during each experiment. The temperature variance within each day was less than 0.25°C for 99% of the recorded days. As expected, widths of head capsules and average larval instar increased with time (Fig. 5.3) as indicated by the significant differences in widths of head capsules among samples (Table 5.1). The duration from egg (start date: 3.5 days after flooding) to the date at which 50% of weevils were in the pupal stage was 19 days for all three experiments (Fig. 5.4). In the third experiment, the duration for pupal development was estimated as 13 days (from 50% pre-pupa to 50% post-pupa). Significant differences was not found in duration of larval development between the two rice varieties (Table 5.1).

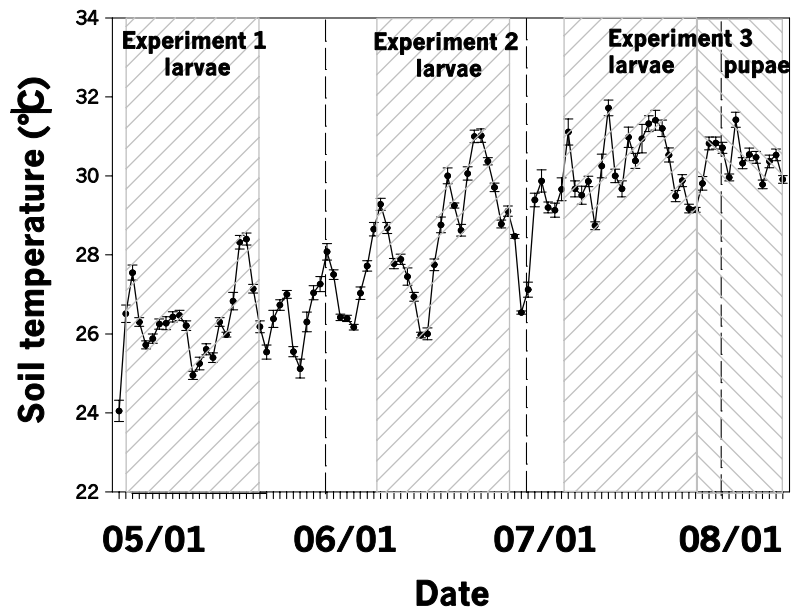
Using a temperature threshold of 10°C, the number of degree-days required for development from egg to 50% pupation was  $359.1 \pm 19.4$  degree-days ( $^{\circ}\text{C} \times \text{Day}$ ), and the number of degree days required for pupal development was 264.3 degree-days ( $^{\circ}\text{C} \times \text{Day}$ ). The total degree-days required for the development of one generation is about 623.4 degree-days ( $^{\circ}\text{C} \times \text{Day}$ ).

### **Discussion**

Degree-day models were developed to describe emergence of weevils from overwintering in early spring and the duration of immature stages on rice roots in



**Figure 5.1** Predicted and actual dates of emergence of rice water weevils from overwintering sites over 17 years, Crowley, Acadia Parish, Louisiana. Predicted dates of emergence are based on a degree-day model developed using emergence and weather data from 1979-1995

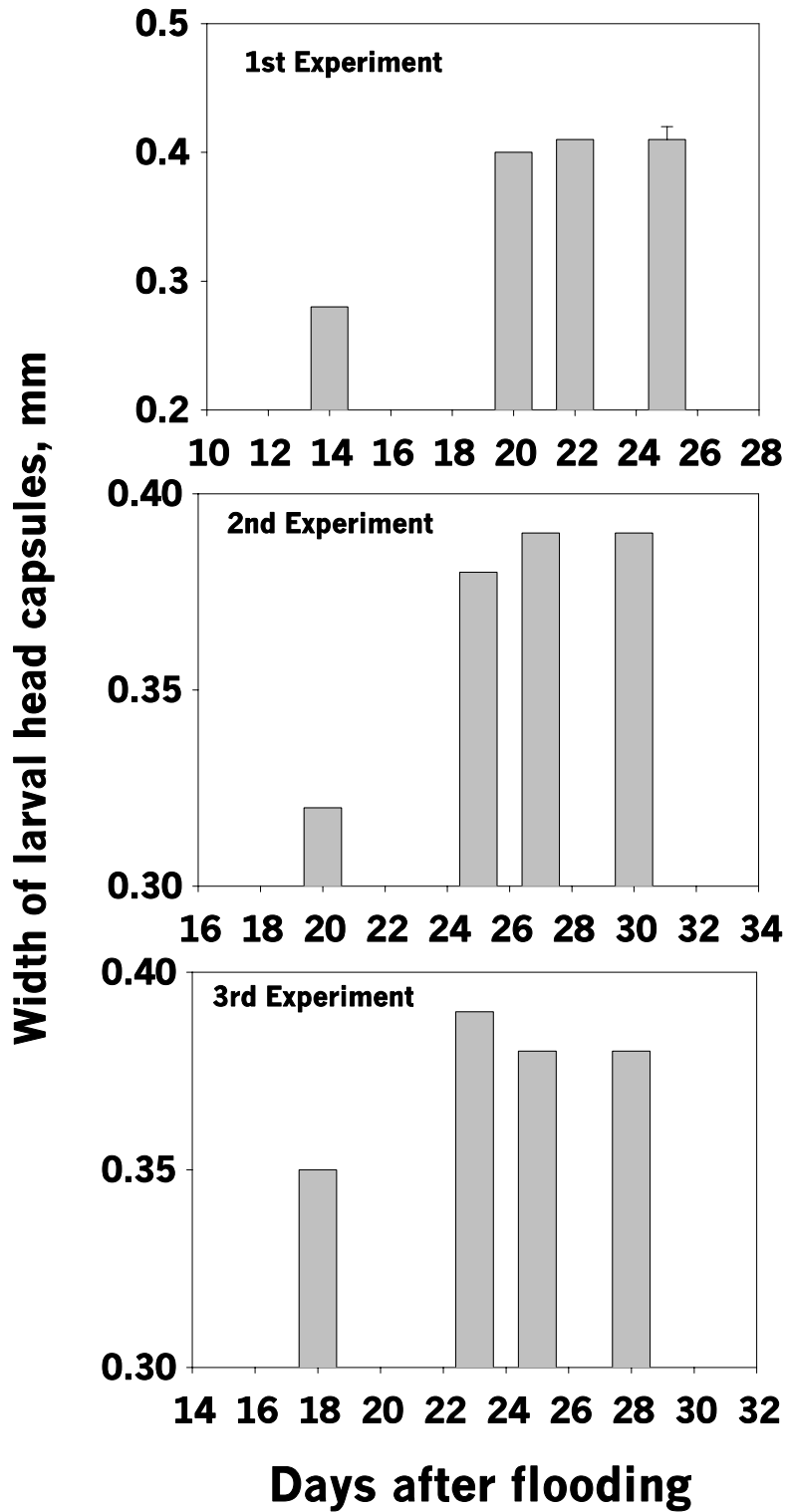


**Figure 5.2** Mean daily soil temperatures ( $\pm$  S.E.) in flooded rice fields, May to August, 2003, Crowley, Acadia Parish, Louisiana. Temperatures were obtained from plots of rice in which rice water weevil development was monitored. Shaded areas show the time period during which larval or pupal development was monitored.

**Table 5. 1** Results of mixed-model analyses of head-capsule development of larvae on rice varieties “Cocodrie” and “Bengal”.

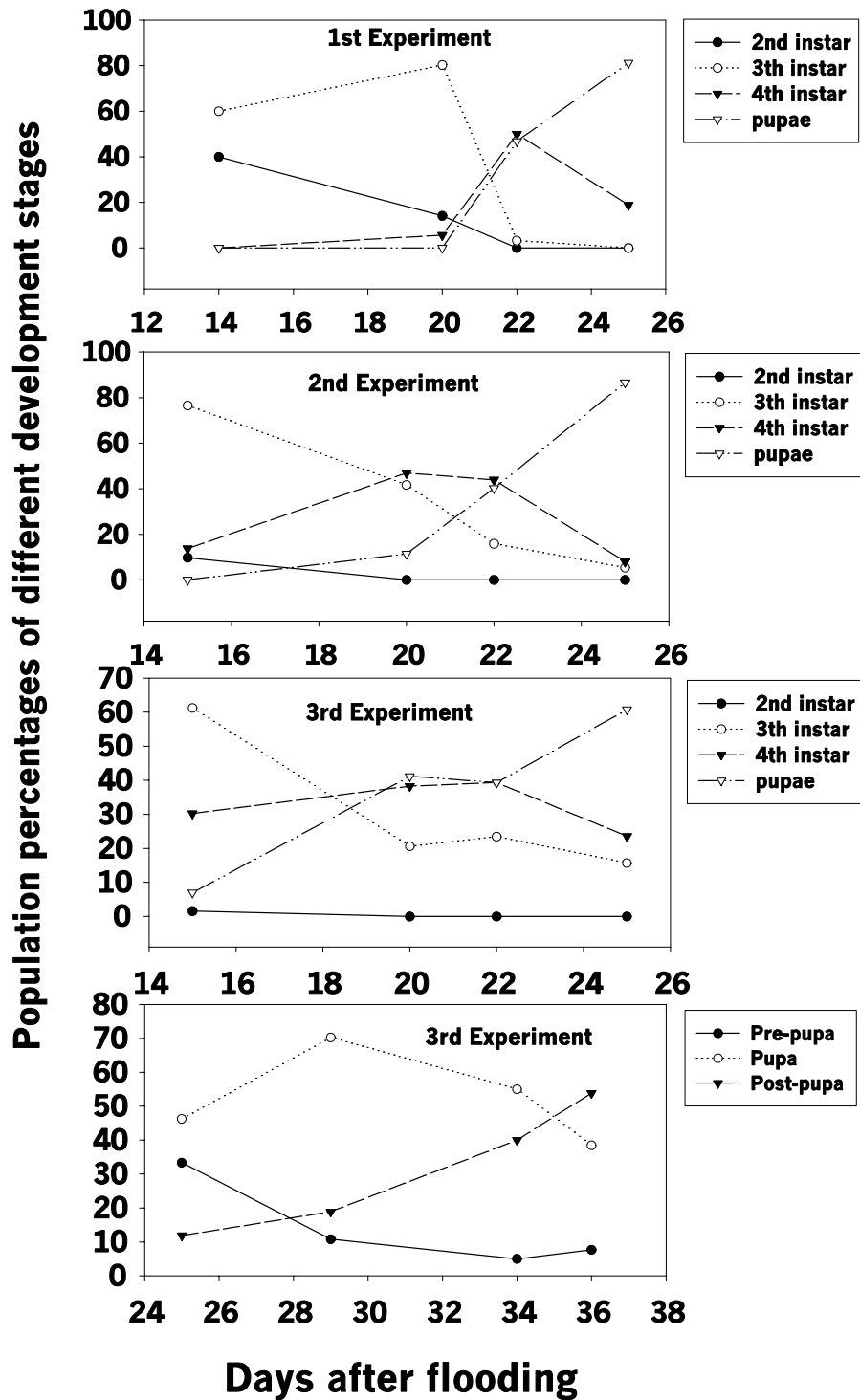
Source of variation	1st Experiment		2 <sup>nd</sup> Experiment		3 <sup>rd</sup> Experiment	
	df	F	df	F	df	F
Variety (Va)	—	—	1	0.16	1	0.27
Sampling Time (St)	3	192.02*	3	119.79*	3	27.84*
Va × St	—	—	3	5.78*	3	1.46

\*  $P < 0.001$



**Figure 5.3** Mean width of larval head capsules as a function of time (days after flooding), Crowley, Acadia Parish, Louisiana. Standard errors for almost all means were less than 0.001mm and were not depicted in figure.





**Figure 5.4** Percentage of larvae on rice roots of various stages as a function of time (days after flooding) in three experiments, Crowley, Acadia Parish, Louisiana. Pupal development was monitored only in the third experiment.

flooded soils. The degree-day model for emergence of weevils from overwintering was used to predict the emergence dates in 2001 through 2003 with acceptable accuracy (on average, within 4 days). The larval development study documented the development of the larval and pupal stages in rice fields for the first time. The total development period for larvae and pupae on rice root systems in this study was about 32 days. The development periods from all three experiments were similar because soil temperatures for rice flooded in May, Jun and July were fairly similar (Fig. 5.2). These two models developed here will facilitate the development of an IPM program for the rice water weevil in Louisiana.

The degree-day model for emergence of weevils will perhaps enable an early prediction system to be established using local meteorological forecast data, although the model needs to be verified in other rice growing regions. Predictions of emergence dates may facilitate the use of early planting as a control tactic. Early planting has been shown to reduce losses from the rice water weevils (Thompson et al. 1994, Stout unpublished data), but the practice is not currently recommended to Louisiana farmers.

Recommended planting dates can be developed based on this model which will allow rice plants to escape heavy infestation at early stages of rice growth (rice plants suffer greater yield losses when they are infested early in their development (Wu and Wilson 1997, Stout et al. 2002b). Predictions made on the basis of this model may also aid the use of adulticidal insecticides against the rice water weevil by alerting farmers to the likely presence of adult weevils in their fields.

Two earlier quantitative or semi-quantitative models were developed to describe emergence of rice water weevils from overwintering (Morgan et al. 1984, Muegge et al.

1996). Morgan et al. (1984) developed a degree-day model to predict cumulative percent weevil emergence in Arkansas, but this model was better suited to predict cumulative emergence over an entire spring than it was to predict initial emergence. The empirical observation of Muegge et al. (1996) that “adults emerge a few days after mean temperatures exceed 60°F (15.6°C)” is less quantitative and reliable than the present model. In 2001, 2002 and 2003, the actual emergence dates varied from 4 to 20 days after mean temperatures exceeded 15.6°C. Research to further validate the current model in North Louisiana and other southern rice growing regions continues.

The quantitative description of larval development also provides valuable information for a weevil management program. Knowledge of the period during which rice is injured by weevil larvae is critical to the design of an IPM program. The most injuring stages of larvae are the 3<sup>rd</sup> and 4<sup>th</sup> instars (Wu and Wilson 1997, Stout et al. 2001). In this study, densities of 3<sup>rd</sup> and 4<sup>th</sup> instars peaked at 14 and 20 days after flooding (Fig. 5.4). The presence of injuring stages of larvae within 2 weeks of flooding is earlier than suggested by previous studies (Cave et al. 1984, Barbour and Muegge 1995). The current study gives a clearer picture of the duration of larval and pupal development because oviposition was confined to a one-week period after flooding by the use of insecticides, something that was not done in previous studies (Cave et al. 1984, Barbour and Muegge 1995). This method allowed the development of a cohort of weevils to be followed more easily. The results of this study highlight the risks involved in flooding rice when it is very young, a common practice in southwest Louisiana. Early flooding of rice plants triggers early infestations and (according to this study) early injury to rice plants. Stout et al. (2002b) and Zou et al. (2004a) provided evidence that larval feeding on young rice

plants with small root systems has greater impact on rice growth and yields than larval feeding on older plants.

In addition, understanding the duration of development of a weevil generation will help us understand the relative timing of appearance of overwintered and first generation weevils in Louisiana rice. This is important because the behavior of overwintered weevils may be different from the behavior of weevils of subsequent generations. In China, the first generation of *L. oryophilus* migrates out of the rice field regardless of whether suitable food resources are abundant (Zhai, B., Nanjing Agricultural University, P.R. China, personal communication). The degree-day model obtained from this study can be used to estimate when generations will occur each year. Different management practices may be developed for later generations of weevils in future studies.

Understanding larval development on different varieties may help elucidate mechanisms of host plant resistance in rice to the rice water weevil. “Jefferson” consistently supports lower densities of larvae and suffers lower yield losses than “Bengal” in field experiments (Stout et al. 2001). Resistance in “Jefferson” was manifested as antixenosis (Stout and Riggio 2002). In this study, differential development on “Jefferson” and “Bengal” would have provided evidence of antibiosis. However, the similarity of immature development on “Jefferson” and “Bengal” indicates that antibiosis is not responsible for lower larval densities on “Jefferson”.

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## CHAPTER 6

### SUMMARY AND CONCLUSION

The rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, is the most destructive insect pest of rice in the United States. As part of developing a sustainable management program for the rice water weevil, these studies were initiated to test the hypothesis that significant benefits can be achieved by the combined use of rice varieties with low levels of host plant resistance and judicious use of cultural practices and insecticides. This study focuses on the use of tolerant varieties because tolerance was expressed consistently by some varieties over two years (Stout et al. 2001). Two complementary approaches, modeling and experimental trials, were used to demonstrate and evaluate the benefits of host plant resistance and other management strategies. Modeling was used to quantify relevant behavioral events in the life history of the rice water weevil. Experimental trials were used to document yield differences between tolerant and susceptible rice varieties over multiple years, growth and yield responses of rice plants to root damage, and weevil density-rice yield relationships under different water management regimes.

Two commercial varieties that had previously shown differences in tolerance to injury by the rice water weevil were evaluated under different management practices for multiple years (Chapter 2). “Cocodrie” is currently the most widely-grown long-grain variety and “Bengal” is the most widely-grown medium-grain variety. Tolerance in “Cocodrie” relative to “Bengal” was manifested consistently over multiple years. Larval densities were similar between these two varieties; however, yield losses for “Cocodrie” were 5-15% less than for “Bengal”. For the reduced insecticide treatment (single



application of lambda-cyhalothrin at 0.034 kg AI ha<sup>-1</sup>) the yield losses relative to yields from fipronil-treated plots was 3% for “Cocodrie” and 8% for “Bengal”. These results validate the use of “Bengal” as an example of a susceptible variety and “Cocodrie” as an example of a variety with low levels of tolerance in the experiments described in Chapters 3 and 4.

The effects of root feeding by larvae of the rice water weevil, on the growth and yield components of rice, *Oryza sativa*, were evaluated using four varieties of rice, “Cocodrie”, “Cypress”, “Bengal”, and “XP1003”, over two years (Chapter 3). Feeding by *L. oryzaophilus* larvae caused extensive damage to root systems. Pruning of root systems resulted in a decrease in tiller number and shoot biomass of rice plants in the vegetative stage of growth. Yield losses were due to a combination of decreases in panicle densities, numbers of grains per panicle, and grain weights. Decreases in panicle densities were a direct result of the reductions in tiller numbers. Reductions in numbers of grains per panicle and grain weights probably resulted from decreases in shoot biomass. Injury by rice water weevil larvae is chronic. The tillering stage of rice suffered the majority of weevil damage, but the growth effects were not manifested until later. This experiment represents a first step toward an in-depth understanding of the mechanism of yield loss from the rice water weevil in rice. Such an understanding will facilitate efforts to breed lines more tolerant of weevil feeding than existing lines. Breeding for tolerance of weevil injury may be a more attainable target than breeding for antibiosis or antixenosis. Results from this study may lead to the identification of physiological traits associated with tolerance. These traits can be targeted or used as markers during breeding. The current procedure for assessing tolerance (determining

differences in larval densities and yields from field plots treated and not treated with insecticides) is a laborious, expensive, and lengthy procedure. Physiological markers or targets for breeding should be helpful to breeding efforts worldwide where this insect is a pest (North America and Asia).

Two years of field experiments were conducted to quantify relationships between densities of immature rice water weevils and yields for two rice varieties under early and delayed flooding regimes. Larval densities during earlier stages of rice growth were more strongly correlated with yield losses than were larval densities later in the growing season. Slopes of regression models were more negative for early-flood than for delayed-flood plots, and more negative for 'Bengal' than for 'Cocodrie'. The results of these experiments confirm that short delays in flooding reduce yield losses from the rice water weevil and that 'Bengal' is less tolerant of weevil feeding than 'Cocodrie'. Estimates of yield loss caused by each weevil provide a key parameter for the calculation of economic injury levels under different management regimes. Data from this study also suggest that expression of tolerance depends on environment: yield losses for both 'Cocodrie' and 'Bengal' were similar under the early-flooded regime, but 'Cocodrie' was clearly more tolerant under a delayed-flood regime. In other words, delayed-flood enhanced the expression of host plant resistance (tolerance).

Degree-day models were developed to predict the emergence date of adults from overwintering in spring and to describe larval and pupal development on two varieties of rice under field conditions. For the overwintering emergence model, the model parameters that gave the minimum error between predicted and actual dates of emergence were: low temperature threshold for flight muscle development, 60 °F; cumulative catch

of weevils, 6; start date for accumulation of degree days, 33 days after January 1<sup>st</sup>. Using these parameters, emergence of weevils occurred after the accumulation of 139.2 degree-days ( $^{\circ}\text{C} \times \text{Day}$ ). For the larval development study, using a temperature threshold of  $10^{\circ}\text{C}$ , the number of degree days required for development from egg to 50% pupation was  $359.11 \pm 19.44$  ( $^{\circ}\text{C} \times \text{Day}$ ), and number of degree days required for pupal development was 264.26 ( $^{\circ}\text{C} \times \text{Day}$ ). The total degree-days required for development of one generation is about 623.37 ( $^{\circ}\text{C} \times \text{Day}$ ). Larval development on varieties “Jefferson” and “Bengal” did not differ. The modeling study provides phenological information that will facilitate the design of an IPM program by helping to schedule planting dates and applications of insecticides. With accurate prediction of the arrival of adults in the field, early planting dates can be recommended which will allow rice plants to grow longer in the absence of weevil feeding, thereby becoming more tolerant to injury of weevils. Field monitoring programs and insecticide application can be pinpointed to target the time period when adults are present in the field.

The benefits of low levels of plant resistance as exemplified by “Cocodrie” versus “Bengal” were analyzed by combining data from multiple experiments. The results of this analysis are shown in Table 6.1. Yield losses were averages from experiments described in Chapters 2, 3, and 4. The average yield was assumed to be 5620 lbs/acre and rice market value was assumed to be \$6 per hundred pounds (figures obtained from Louisiana Agricultural Statistics Service, 2001). The cost of insecticide application was estimated as \$15 (fipronil at 0.0375 kg AI/ha) and \$10 (lambda-cyhalothrin, 0.034 kg AI/ha) (Stout, personal communication). The economic return was calculated using the function, total rice dollar value (yield multiplied by unit price) - insecticide cost - dollar

loss from yield loss. For example, the income for “Tolerant variety + low insecticide” was calculated as:  $\$337 (5620 \text{ lbs/A} * \$0.06/\text{lb}) - \$10 (\text{insecticide cost}) - \$337 * 3\% (\text{yield loss}) = \$317$  (Table 6.1). The highest economic return was given by use of the high rate insecticide (fipronil at  $0.0375 \text{ kg AI ha}^{-1}$ ). The reason for this is that rice is a high value crop and the cost of insecticide was relatively low. Assuming a fixed cost of insecticide,  $\$15/\text{acre}$ , the economic injury level is 4.5% yield loss ( $\$15/\$337$ ). However, the market is dynamic. If rice prices drop and/or insecticide costs increase, the economic injury level will increase. In that case, management strategies should shift towards the use of host plant resistance and cultural practices. In addition, in some circumstances, growers can't use insecticides because of environmental and non-target effects. If a more tolerant variety can be developed, the economic benefits of using tolerant rice varieties will be greater.

In conclusion, this study showed that the use of host-plant resistance is compatible with the use of management practices such as delayed-flooding, early planting, and reduced use of insecticides. These management tactics are compatible with each other. Although use of high rate of insecticide currently gives the best economic return, integrated control provides a combination of adequate control and environmental friendliness. Similar economic returns can be achieved using a more tolerant variety and delayed-flood without insecticide treatment. In the long term, an integrated management system can be built by combining accurate prediction of rice water weevil population dynamics, selection of tolerant varieties, use of early planting or delayed-flooding, and judicious use of insecticides.

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**Table 6. 1.** Economic comparison of using different management tactics

Management tactics	Yield loss <sup>1</sup> (%/acre)	Dollar loss from yield loss <sup>2</sup> (\$/acre)	Treatment cost <sup>3</sup> (\$/acre)	Net income <sup>4</sup> (\$/acre)
High insecticide (fipronil, 0.0375 kg AI/ha)	0	0	15	322
Tolerant variety + low insecticide (lambda-cyhalothrin, 0.034 kg AI/ha)	3	10	10	317
Tolerant variety + delayed-flood	10	34	0	303
Susceptible variety + low insecticide (lambda-cyhalothrin, 0.034 kg AI/ha)	8	27	10	300
Tolerant variety + early-flood or Susceptible variety + delayed-flood	19	64	0	273
Susceptible variety + early-flood	27	91	0	246

<sup>1</sup>Relative to high insecticide treatment (0.0375 kg fipronil ha<sup>-1</sup>)

<sup>2</sup>Dollar loss from yield loss = total rice dollar value \* yield loss (%). Average yield = 5620 lbs per acre, rice market value = \$6 per 100lbs (Louisiana Agricultural Statistics Service, 2001). Total rice dollar value = \$337 (5620\*6/100).

<sup>3</sup>High insecticide cost = \$15 per acre, low insecticide cost = \$10 per acre.

<sup>4</sup>Net income = total rice dollar value - insecticide cost - dollar loss from yield loss.

## APPENDIX A. PERMISSION FROM JOURNALS

### Letter from Dr. Steve Naranjo, Chief Editor of "Crop Protection"

From: Steve Naranjo <snaranjo@wcr1.ars.usda.gov> on 03/02/2004 09:25 AM MST  
Sent by: Steve Naranjo [snaranjo@wcr1.ars.usda.gov](mailto:snaranjo@wcr1.ars.usda.gov)  
To: Li Zou <lzou1@lsu.edu>cc:  
Subject: Re: permission

Li

According to what I have been able to glean from the Elsevier website you are automatically granted permission to reproduce an article in your dissertation. Please see <http://authors.elsevier.com/Look> under Copyright Information - What rights do I retain as an author - 8th point down. It would still be a good idea to acknowledge that the paper is "in press" with Crop Protection. If you still need some sort of letter let me know.

Steve

### Letter from Dr. Allan Watt, Chief Editor of "Agricultural and Forest Entomology"

Sent by: "Allan Watt" <adw@ceh.ac.uk>  
To: <lzou1@lsu.edu>  
cc:  
Subject: Agricultural and Forest Entomology  
Dear Li,

As editor of Agricultural and Forest Entomology I grant permission to you to publish the paper "The effects of feeding by the rice water weevil on the growth and yield", which will appear in the Journal, in your dissertation.

And good luck!!

Yours sincerely,

Allan Watt

-----  
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## APPENDIX B. SAS PROGRAM

### Example: Density-yield model selection for 2002

```
/*DENSITY YIELD 2002
EARLY FLOOD L1(20D) L2(32D)
LATE FLOOD L1(24D) L2(33D) */
DATA YIELD;
INFILE CARDS MISSOEVER;
INPUT FLOOD$ TREAT$ PLOT$ VARIETY$ BLOCK$ UNJYIELD MOIST L1 L2;
ADJYIELD=(( (100-MOIST)/88)*UNJYIELD)*595.74;

/*UNDAMAGED YIELD IN LATE FLOODED PLOTS FOR COCODRIE*/
YLC1=(( (100-18.5)/88)*14.2)*595.74;
YLC2=(( (100-18.8)/88)*15.1)*595.74;
YLC3=(( (100-18.9)/88)*15.1)*595.74;
YLC4=(( (100-19.2)/88)*14.8)*595.74;
/*UNDAMAGED YIELD IN LATE FLOODED PLOTS FOR BENGAL*/

YLB1=(( (100-19.2)/88)*13.9)*595.74;
YLB2=(( (100-19.1)/88)*13.5)*595.74;
YLB3=(( (100-19.5)/88)*14.0)*595.74;
YLB4=(( (100-20.0)/88)*13.8)*595.74;

/*UNDAMAGED YIELD IN EARLY FLOODED PLOTS FOR COCODRIE*/
YEC1=(( (100-16.9)/88)*12.4)*595.74;
YEC2=(( (100-16.7)/88)*12.7)*595.74;
YEC3=(( (100-16.9)/88)*12.7)*595.74;
YEC4=(( (100-16.7)/88)*12.8)*595.74;

/*UNDAMAGED YIELD IN EARLY FLOODED PLOTS FOR BENGAL*/
YEB1=(( (100-17.4)/88)*11.3)*595.74;
YEB2=(( (100-17.5)/88)*10.9)*595.74;
YEB3=(( (100-17.3)/88)*11.9)*595.74;
YEB4=(( (100-17.4)/88)*11.7)*595.74;

/*PERCENTAGE YIELD*/
IF FLOOD='L' AND VARIETY='COC' AND BLOCK='1' THEN PYIELD=ADJYIELD/YLC1;
IF FLOOD='L' AND VARIETY='COC' AND BLOCK='2' THEN PYIELD=ADJYIELD/YLC2;
IF FLOOD='L' AND VARIETY='COC' AND BLOCK='3' THEN PYIELD=ADJYIELD/YLC3;
IF FLOOD='L' AND VARIETY='COC' AND BLOCK='4' THEN PYIELD=ADJYIELD/YLC4;
IF FLOOD='L' AND VARIETY='BEN' AND BLOCK='1' THEN PYIELD=ADJYIELD/YLB1;
IF FLOOD='L' AND VARIETY='BEN' AND BLOCK='2' THEN PYIELD=ADJYIELD/YLB2;
IF FLOOD='L' AND VARIETY='BEN' AND BLOCK='3' THEN PYIELD=ADJYIELD/YLB3;
IF FLOOD='L' AND VARIETY='BEN' AND BLOCK='4' THEN PYIELD=ADJYIELD/YLB4;
IF FLOOD='E' AND VARIETY='COC' AND BLOCK='1' THEN PYIELD=ADJYIELD/YEC1;
IF FLOOD='E' AND VARIETY='COC' AND BLOCK='2' THEN PYIELD=ADJYIELD/YEC2;
IF FLOOD='E' AND VARIETY='COC' AND BLOCK='3' THEN PYIELD=ADJYIELD/YEC3;
IF FLOOD='E' AND VARIETY='COC' AND BLOCK='4' THEN PYIELD=ADJYIELD/YEC4;
IF FLOOD='E' AND VARIETY='BEN' AND BLOCK='1' THEN PYIELD=ADJYIELD/YEB1;
IF FLOOD='E' AND VARIETY='BEN' AND BLOCK='2' THEN PYIELD=ADJYIELD/YEB2;
IF FLOOD='E' AND VARIETY='BEN' AND BLOCK='3' THEN PYIELD=ADJYIELD/YEB3;
IF FLOOD='E' AND VARIETY='BEN' AND BLOCK='4' THEN PYIELD=ADJYIELD/YEB4;
PLOSS=1-PYIELD;
TOTAL=L1+L2;
AVG=TOTAL/2;
```

```

L1S=L1*L1;
L1Q=L1S*L1;
L2S=L2*L2;
L2Q=L2S*L2;
TOTALS=TOTAL*TOTAL;
TOTALQ=TOTALS*TOTAL;
CARDS;
L 4 101 COC 1 12.4 18.7 43.3 24.3
L 6 102 COC 1 14.2 18.5 19.7 21
L 2 103 COC 2 12.8 18.7 43 27.3
L 4 104 BEN 2 10.8 19.4 25 26.7
L 4 105 COC 3 13.0 18.8 17.3 38.3
L 3 106 BEN 3 10.9 20.8 31 35.7
L 5 107 COC 4 13.7 18.7 13.3 26
L 1 108 BEN 4 9.7 20.0 38.7 27
L 2 201 COC 1 13.7 18.4 31.3 20.7
L 4 202 BEN 1 11.5 19.1 33 28
L 6 203 BEN 2 13.5 19.1 12.3 18
L 4 204 COC 2 13.6 18.4 41 26.7
L 6 205 COC 3 15.1 18.9 7.7 21
L 5 206 COC 3 14.7 18.7 31 32
L 3 207 BEN 4 11.3 19.2 39 21
L 3 208 COC 4 13.2 18.3 31.3 31.7
L 3 301 COC 1 13.0 18.6 27.3 18.7
L 3 302 BEN 1 11.8 18.8 10.7 24.3
L 5 303 BEN 2 12.2 18.7 22.7 22.3
L 2 304 BEN 2 11.0 19.2 36 15.3
L 3 305 COC 3 12.6 18.3 23 19
L 1 306 BEN 3 10.5 20.6 46.3 25
L 1 307 COC 4 12.0 18.2 40.3 40
L 4 308 COC 4 14.1 18.4 29.3 20.7
L 6 401 BEN 1 13.9 19.2 6.7 14.7
L 1 402 BEN 1 10.8 20.6 30.3 40
L 3 403 COC 2 13.8 18.5 21 29
L 6 404 COC 2 15.1 18.8 3 14
L 1 405 COC 3 12.1 18.3 33 39.7
L 2 406 BEN 3 9.6 20.2 23.3 55
L 6 407 BEN 4 13.8 20.0 7.7 7
L 6 408 COC 4 14.8 19.2 2.3 10.3
L 1 501 COC 1 11.8 18.5 33 52.3
L 2 502 BEN 1 9.8 20.3 23.7 33.7
L 1 503 COC 2 12.3 18.7 42.3 50.7
L 5 504 COC 2 13.8 18.8 15 20
L 5 505 BEN 3 11.2 18.4 13 33.7
L 2 506 COC 3 11.0 18.2 30.7 32.7
L 2 507 BEN 4 9.4 18.6 36 31
L 2 508 COC 4 11.8 19.0 34.7 39.3
L 5 601 COC 1 13.7 18.8 23.3 27.3
L 5 602 BEN 1 11.2 19.1 19.7 35.3
L 3 603 BEN 2 9.3 18.4 25 42
L 1 604 BEN 2 10.4 18.9 33.7 56.3
L 4 605 BEN 3 9.5 18.3 31 44.3
L 6 606 BEN 3 14.0 19.5 4.3 15
L 4 607 BEN 4 9.9 18.1 30.7 42.7
L 5 608 BEN 4 11.6 19.1 16 25.7
E 2 101 COC 1 6.2 17.2 22.7 28
E 4 102 COC 1 9.3 16.7 12 23.3

```

```

E 5 103 BEN 2 10.5 17.1 10.7 12.7
E 2 104 BEN 2 6.3 18.4 24 12.7
E 2 105 BEN 3 6.9 19.2 12 30.3
E 2 106 COC 3 6.4 17.1 37 16
E 3 107 BEN 4 8.6 17.4 16.3 27
E 5 108 BEN 4 9.6 17.3 6 9.3
E 5 201 COC 1 11.1 16.8 13.3 23.3
E 3 202 BEN 1 8.1 17.6 20.7 28.7
E 5 203 COC 2 11.9 16.7 10.3 18
E 4 204 BEN 2 9.4 17.6 8.3 21.3
E 1 205 COC 3 7.0 17.1 42.3 32.3
E 3 206 COC 3 8.8 16.8 32.3 35.7
E 6 207 BEN 4 11.7 17.4 2.7 6
E 4 208 COC 4 10.1 16.7 20.3 33.3
E 4 301 BEN 1 9.3 17.4 12.3 18.3
E 6 302 BEN 1 11.3 17.4 12 6.3
E 3 303 BEN 2 9.8 17.7 11.3 14.7
E 6 304 COC 2 12.7 16.7 3.3 7.7
E 4 305 COC 3 10.7 16.5 6 21.7
E 1 306 BEN 3 6.4 19.3 32 19.7
E 1 307 COC 4 5.8 17.2 24.3 17.7
E 4 308 BEN 4 9.3 17.8 11.7 13.3
E 1 401 COC 1 5.6 17.4 39.3 37
E 1 402 BEN 1 5.5 19.3 40 24.3
E 4 403 COC 2 10.5 17.0 20.3 26
E 1 404 COC 2 5.7 17.3 32.7 51.3
E 5 405 BEN 3 11.0 17.2 4 15.7
E 4 406 BEN 3 9.4 17.4 16 34.7
E 1 407 BEN 4 5.7 20.4 46.7 35
E 2 408 BEN 4 6.6 18.5 33.3 27.7
E 2 501 BEN 1 7.7 19.0 30.7 33.7
E 3 502 COC 1 9.9 16.7 18.7 41.7
E 2 503 COC 2 9.7 16.8 23 43
E 3 504 COC 2 11.1 16.8 12.3 34.3
E 6 505 BEN 3 11.9 17.3 4 5.7
E 5 506 COC 3 12.6 16.6 14.7 7.7
E 5 507 COC 4 11.2 17.0 14 17
E 2 508 COC 4 8.2 17.0 50.7 33.7
E 6 601 COC 1 12.4 16.9 4.7 13.7
E 5 602 BEN 1 10.9 17.3 4.7 14
E 6 603 BEN 2 10.9 17.5 7.7 10.2
E 1 604 BEN 2 5.8 19.9 36.3 34.7
E 3 605 BEN 3 9.3 17.4 18.7 36
E 6 606 COC 3 12.7 16.9 2.3 10.3
E 6 607 COC 4 12.8 16.7 3.7 7
E 3 608 COC 4 9.0 16.7 12 48.7

```

**RUN;**

```

/*GENERATE DATASETS FOR VARIETY AND FLOOD COMBINATIONS*/
DATA YIELDE YIELDL YIELDLDC YIELDDB YIELDDEC YIELDEB YIELDLC YIELDLB;
SET YIELD;
IF FLOOD="E" THEN OUTPUT YIELDE;
IF FLOOD="L" THEN OUTPUT YIELDL;
IF VARIETY="COC" THEN OUTPUT YIELDLDC;
IF VARIETY="BEN" THEN OUTPUT YIELDDB;
IF FLOOD="E" AND VARIETY='COC' THEN OUTPUT YIELDDEC;
IF FLOOD="E" AND VARIETY='BEN' THEN OUTPUT YIELDEB;

```

```

IF FLOOD="L" AND VARIETY='COC' THEN OUTPUT YIELDLC;
IF FLOOD="L" AND VARIETY='BEN' THEN OUTPUT YIELDLB;
RUN;

PROC SORT DATA=YIELD;
BY TREAT VARIETY;
RUN;
PROC MEANS DATA=YIELD MEAN STDERR ;
VAR PLOSS; BY TREAT VARIETY;
RUN;
PROC SORT DATA=YIELDL;
BY VARIETY;
RUN;
PROC MEANS DATA=YIELDL MEAN STDERR ;
VAR ADJYIELD; BY VARIETY;
RUN;

TITLE 'EXAM ALL POSSIBLE AND SELECT CANDIDATE MODELS';
PROC RSQUARE DATA=YIELDLC CP MSE;
RICKER:MODEL PYIELD=L2 L2S L2Q;
RUN;

TITLE 'MAKING SELECTION FROM CANDIDATE MODELS';

PROC REG DATA=YIELDLC;

TITLE 'PARAMETER ESTIMATE OF EARLY-FLOOD, SAMPLE_20D';

YLA1:MODEL PYIELD=L2 ;ID PYIELD L2;
RESTRICT INTERCEPT=1;
OUTPUT OUT=PY1 P=PT R=R1 PRESS=PRESSU;

YLA12:MODEL PYIELD=L2 L2S;ID PYIELD L2;
RESTRICT INTERCEPT=1;
OUTPUT OUT=PY12 P=PT R=R1 PRESS=PRESSU;

YLA123:MODEL PYIELD=L2 L2S L2Q;ID PYIELD L2;
RESTRICT INTERCEPT=1;
OUTPUT OUT=PY123 P=PT R=R1 PRESS=PRESSU;
RUN;

```

### Example: Mixed Model

```

/*DENSITY YIELD 2002
EARLY FLOOD L1(20D) L2(32D)
LATE FLOOD L1(24D) L2(33D)
*/
DATA YIELD;
INFILE CARDS MISSOEVER;
INPUT FLOOD$ TREAT$ PLOT$ VARIETY$ BLOCK$ UNJYIELD MOIST L1 L2;
ADJYIELD=(( (100-MOIST)/88)*UNJYIELD)*595.74;

/*UNDAMAGED YIELD IN LATE FLOODED PLOTS FOR COCODRIE*/
YLC1=(( (100-18.5)/88)*14.2)*595.74;
YLC2=(( (100-18.8)/88)*15.1)*595.74;

```

```

YLC3=(( (100-18.9)/88)*15.1)*595.74;
YLC4=(( (100-19.2)/88)*14.8)*595.74;
/*UNDAMAGED YIELD IN LATE FLOODED PLOTS FOR BENGAL*/

YLB1=(( (100-19.2)/88)*13.9)*595.74;
YLB2=(( (100-19.1)/88)*13.5)*595.74;
YLB3=(( (100-19.5)/88)*14.0)*595.74;
YLB4=(( (100-20.0)/88)*13.8)*595.74;

/*UNDAMAGED YIELD IN EARLY FLOODED PLOTS FOR COCODRIE*/
YEC1=(( (100-16.9)/88)*12.4)*595.74;
YEC2=(( (100-16.7)/88)*12.7)*595.74;
YEC3=(( (100-16.9)/88)*12.7)*595.74;
YEC4=(( (100-16.7)/88)*12.8)*595.74;

/*UNDAMAGED YIELD IN EARLY FLOODED PLOTS FOR BENGAL*/
YEB1=(( (100-17.4)/88)*11.3)*595.74;
YEB2=(( (100-17.5)/88)*10.9)*595.74;
YEB3=(( (100-17.3)/88)*11.9)*595.74;
YEB4=(( (100-17.4)/88)*11.7)*595.74;

/*PERCENTAGE YIELD*/
IF FLOOD='L' AND VARIETY='COC' AND BLOCK='1' THEN PYIELD=ADJYIELD/YLC1;
IF FLOOD='L' AND VARIETY='COC' AND BLOCK='2' THEN PYIELD=ADJYIELD/YLC2;
IF FLOOD='L' AND VARIETY='COC' AND BLOCK='3' THEN PYIELD=ADJYIELD/YLC3;
IF FLOOD='L' AND VARIETY='COC' AND BLOCK='4' THEN PYIELD=ADJYIELD/YLC4;
IF FLOOD='L' AND VARIETY='BEN' AND BLOCK='1' THEN PYIELD=ADJYIELD/YLB1;
IF FLOOD='L' AND VARIETY='BEN' AND BLOCK='2' THEN PYIELD=ADJYIELD/YLB2;
IF FLOOD='L' AND VARIETY='BEN' AND BLOCK='3' THEN PYIELD=ADJYIELD/YLB3;
IF FLOOD='L' AND VARIETY='BEN' AND BLOCK='4' THEN PYIELD=ADJYIELD/YLB4;
IF FLOOD='E' AND VARIETY='COC' AND BLOCK='1' THEN PYIELD=ADJYIELD/YEC1;
IF FLOOD='E' AND VARIETY='COC' AND BLOCK='2' THEN PYIELD=ADJYIELD/YEC2;
IF FLOOD='E' AND VARIETY='COC' AND BLOCK='3' THEN PYIELD=ADJYIELD/YEC3;
IF FLOOD='E' AND VARIETY='COC' AND BLOCK='4' THEN PYIELD=ADJYIELD/YEC4;
IF FLOOD='E' AND VARIETY='BEN' AND BLOCK='1' THEN PYIELD=ADJYIELD/YEB1;
IF FLOOD='E' AND VARIETY='BEN' AND BLOCK='2' THEN PYIELD=ADJYIELD/YEB2;
IF FLOOD='E' AND VARIETY='BEN' AND BLOCK='3' THEN PYIELD=ADJYIELD/YEB3;
IF FLOOD='E' AND VARIETY='BEN' AND BLOCK='4' THEN PYIELD=ADJYIELD/YEB4;
PLOSS=1-PYIELD;
TOTAL=L1+L2;
AVG=TOTAL/2;
LARVAE=L1;SAMPLE='S1';OUTPUT;
LARVAE=L2;SAMPLE='S2';OUTPUT;
CARDS;
L 4 101 COC 1 12.4 18.7 43.3 24.3
L 6 102 COC 1 14.2 18.5 19.7 21
L 2 103 COC 2 12.8 18.7 43 27.3
L 4 104 BEN 2 10.8 19.4 25 26.7
L 4 105 COC 3 13.0 18.8 17.3 38.3
L 3 106 BEN 3 10.9 20.8 31 35.7
L 5 107 COC 4 13.7 18.7 13.3 26
L 1 108 BEN 4 9.7 20.0 38.7 27
L 2 201 COC 1 13.7 18.4 31.3 20.7
L 4 202 BEN 1 11.5 19.1 33 28
L 6 203 BEN 2 13.5 19.1 12.3 18
L 4 204 COC 2 13.6 18.4 41 26.7
L 6 205 COC 3 15.1 18.9 7.7 21

```

L 5 206 COC 3 14.7 18.7 31 32  
L 3 207 BEN 4 11.3 19.2 39 21  
L 3 208 COC 4 13.2 18.3 31.3 31.7  
L 3 301 COC 1 13.0 18.6 27.3 18.7  
L 3 302 BEN 1 11.8 18.8 10.7 24.3  
L 5 303 BEN 2 12.2 18.7 22.7 22.3  
L 2 304 BEN 2 11.0 19.2 36 15.3  
L 3 305 COC 3 12.6 18.3 23 19  
L 1 306 BEN 3 10.5 20.6 46.3 25  
L 1 307 COC 4 12.0 18.2 40.3 40  
L 4 308 COC 4 14.1 18.4 29.3 20.7  
L 6 401 BEN 1 13.9 19.2 6.7 14.7  
L 1 402 BEN 1 10.8 20.6 30.3 40  
L 3 403 COC 2 13.8 18.5 21 29  
L 6 404 COC 2 15.1 18.8 3 14  
L 1 405 COC 3 12.1 18.3 33 39.7  
L 2 406 BEN 3 9.6 20.2 23.3 55  
L 6 407 BEN 4 13.8 20.0 7.7 7  
L 6 408 COC 4 14.8 19.2 2.3 10.3  
L 1 501 COC 1 11.8 18.5 33 52.3  
L 2 502 BEN 1 9.8 20.3 23.7 33.7  
L 1 503 COC 2 12.3 18.7 42.3 50.7  
L 5 504 COC 2 13.8 18.8 15 20  
L 5 505 BEN 3 11.2 18.4 13 33.7  
L 2 506 COC 3 11.0 18.2 30.7 32.7  
L 2 507 BEN 4 9.4 18.6 36 31  
L 2 508 COC 4 11.8 19.0 34.7 39.3  
L 5 601 COC 1 13.7 18.8 23.3 27.3  
L 5 602 BEN 1 11.2 19.1 19.7 35.3  
L 3 603 BEN 2 9.3 18.4 25 42  
L 1 604 BEN 2 10.4 18.9 33.7 56.3  
L 4 605 BEN 3 9.5 18.3 31 44.3  
L 6 606 BEN 3 14.0 19.5 4.3 15  
L 4 607 BEN 4 9.9 18.1 30.7 42.7  
L 5 608 BEN 4 11.6 19.1 16 25.7  
E 2 101 COC 1 6.2 17.2 22.7 28  
E 4 102 COC 1 9.3 16.7 12 23.3  
E 5 103 BEN 2 10.5 17.1 10.7 12.7  
E 2 104 BEN 2 6.3 18.4 24 12.7  
E 2 105 BEN 3 6.9 19.2 12 30.3  
E 2 106 COC 3 6.4 17.1 37 16  
E 3 107 BEN 4 8.6 17.4 16.3 27  
E 5 108 BEN 4 9.6 17.3 6 9.3  
E 5 201 COC 1 11.1 16.8 13.3 23.3  
E 3 202 BEN 1 8.1 17.6 20.7 28.7  
E 5 203 COC 2 11.9 16.7 10.3 18  
E 4 204 BEN 2 9.4 17.6 8.3 21.3  
E 1 205 COC 3 7.0 17.1 42.3 32.3  
E 3 206 COC 3 8.8 16.8 32.3 35.7  
E 6 207 BEN 4 11.7 17.4 2.7 6  
E 4 208 COC 4 10.1 16.7 20.3 33.3  
E 4 301 BEN 1 9.3 17.4 12.3 18.3  
E 6 302 BEN 1 11.3 17.4 12 6.3  
E 3 303 BEN 2 9.8 17.7 11.3 14.7  
E 6 304 COC 2 12.7 16.7 3.3 7.7  
E 4 305 COC 3 10.7 16.5 6 21.7  
E 1 306 BEN 3 6.4 19.3 32 19.7

```

E 1 307 COC 4 5.8 17.2 24.3 17.7
E 4 308 BEN 4 9.3 17.8 11.7 13.3
E 1 401 COC 1 5.6 17.4 39.3 37
E 1 402 BEN 1 5.5 19.3 40 24.3
E 4 403 COC 2 10.5 17.0 20.3 26
E 1 404 COC 2 5.7 17.3 32.7 51.3
E 5 405 BEN 3 11.0 17.2 4 15.7
E 4 406 BEN 3 9.4 17.4 16 34.7
E 1 407 BEN 4 5.7 20.4 46.7 35
E 2 408 BEN 4 6.6 18.5 33.3 27.7
E 2 501 BEN 1 7.7 19.0 30.7 33.7
E 3 502 COC 1 9.9 16.7 18.7 41.7
E 2 503 COC 2 9.7 16.8 23 43
E 3 504 COC 2 11.1 16.8 12.3 34.3
E 6 505 BEN 3 11.9 17.3 4 5.7
E 5 506 COC 3 12.6 16.6 14.7 7.7
E 5 507 COC 4 11.2 17.0 14 17
E 2 508 COC 4 8.2 17.0 50.7 33.7
E 6 601 COC 1 12.4 16.9 4.7 13.7
E 5 602 BEN 1 10.9 17.3 4.7 14
E 6 603 BEN 2 10.9 17.5 7.7 10.2
E 1 604 BEN 2 5.8 19.9 36.3 34.7
E 3 605 BEN 3 9.3 17.4 18.7 36
E 6 606 COC 3 12.7 16.9 2.3 10.3
E 6 607 COC 4 12.8 16.7 3.7 7
E 3 608 COC 4 9.0 16.7 12 48.7

```

**RUN;**

```

/*GENERATE DATASETS FOR VARIETY AND FLOOD COMBINATIONS*/
DATA YIELDE YIELDL YIELDC YIELDB YIELDEC YIELDEB YIELDLB;
SET YIELD;
IF FLOOD="E" THEN OUTPUT YIELDE;
IF FLOOD="L" THEN OUTPUT YIELDL;
IF VARIETY="COC" THEN OUTPUT YIELDC;
IF VARIETY="BEN" THEN OUTPUT YIELDB;
IF FLOOD="E" AND VARIETY='COC' THEN OUTPUT YIELDEC;
IF FLOOD="E" AND VARIETY='BEN' THEN OUTPUT YIELDEB;
IF FLOOD="L" AND VARIETY='COC' THEN OUTPUT YIELDLB;
IF FLOOD="L" AND VARIETY='BEN' THEN OUTPUT YIELDLB;

```

**TITLE 'ANOVA';**

**/\*change YIELDL to YIELDE TO RUN EARLY FLOOD\*/**

```

PROC MIXED DATA=YIELDL;
CLASS BLOCK TREAT VARIETY;
MODEL PLOSS=VARIETY|TREAT/ddfm=satterthwaite;
RANDOM BLOCK BLOCK*VARIETY;

```

```

PROC MIXED DATA=YIELDL;
CLASS BLOCK TREAT VARIETY SAMPLE;
MODEL LARVAE=VARIETY|TREAT|SAMPLE/ddfm=satterthwaite;
RANDOM BLOCK BLOCK*VARIETY TREAT*BLOCK*VARIETY;

```

## APPENDIX C. C PROGRAM OF DEGREE-DAY MODEL

```
/*
Title: Degree Day Model for the Emergence of Rice Water Weevil
Programmer: Li Zou & Michael J. Stout
Time: Feb. 2003
Data source: degreeday folder (5.5M) at Rice Entomology Lab, LSU,
              225-578-1850, A256 Life Science Annex
Compiler: Microsoft Visual C++
Instruction: copy the folder to local C drive. Click degreeday.c
              or run degreeday.exe at c:\degreeday\debug
*/

#include <stdio.h>
#include <stdlib.h>
#include <math.h>

typedef enum {FALSE, TRUE} boolean;

typedef struct degreeday
{
    char date[14];
    double numRww;
    int maxTemp;
    int minTemp;
}DEGREEDAY, *DDPTR;

typedef struct result
{
    int threshold;
    double rwwNumber;
    int startDay;
    double DDF[14];
    double avgDDF;
    int estDDFDay[14];
    int actDDFDay[14];
}RESULT, *RESULTPTR;

typedef struct meanerror
{
    double meanF;
    double rmseF;
}MEANERROR, *MEANERRORPTR;

/*Function Prototypes*/

void openfiles(char *input_file,FILE **inp);
void closefiles(FILE *inp);
void file_specify(char *filename[]);
void read_data(RESULTPTR *result,int heatF,double rwwNumber,int startDay);
void create_degree_data(struct degreeday **dd);
void degree_data_toZero(struct degreeday **dd);
void create_result(struct result **resu);
void create_meanError(struct meanerror **meanerr);
void create_year(char *year[]);
void print_result(RESULTPTR minErrF,MEANERRORPTR meanErr,RESULTPTR predict);

void average_DD(double DDF[],double *avgDDF,int replicate);
void degree_day_DDF(double DD,double *DDF);
void single_sine_lowInter(struct degreeday *dd,double *DD,int heatF);
void intercept_low_threshold(int maxTemp,int minTemp,int heatF,double *DD);
void entirely_between_threshold(int maxTemp,int minTemp,int heatF,double *DD);

void read_data_verify(RESULTPTR *result,int heatF,double rwwNumber,int startDay);
void get_mean_error(MEANERRORPTR *meanErr,RESULTPTR result,int replicate);
void get_minimize_error(RESULTPTR *minErrF,RESULTPTR result,
                        MEANERRORPTR *meanErr,FILE *outp);
void predict_event(RESULTPTR result,RESULTPTR *predict);
```



```

/* Main Drive Function
Input: parameters (temperature threshold, number of weevils caught,
                start dates for accumulating Degree*Day)
Output: parameters with minimum RMSE
Process: call read_data module
        call read_data_verify module
        call get_minimize_error module
        call predict_event module
        call print_result module
        free memory
*/

int main(void)
{
    int lowThreshold=0, rwwNumber=0, startDay=0;
    MEANERRORPTR meanErr=NULL;
    RESULTPTR result=NULL, minErrF=NULL, predict=NULL;
    FILE *outp=NULL;
    char *output_file="C:\\degreeday\\rmsr.txt";
    outp=fopen(output_file, "wt");

    create_meanError(&meanErr);
    create_result(&minErrF);
    create_result(&predict);

    for(lowThreshold=50; lowThreshold<=60; lowThreshold++)
    {
        for(rwwNumber=1; rwwNumber<=10; rwwNumber++)
        {
            for(startDay=30; startDay<=60; startDay++)
            {
                read_data(&result, lowThreshold, rwwNumber, startDay);
                read_data_verify(&result, lowThreshold, rwwNumber, startDay);
                get_minimize_error(&minErrF, result, &meanErr, outp);
            }
        }

        predict_event(minErrF, &predict);

        print_result(minErrF, meanErr, predict);

        return 0;
    }

/*Open File*/
void openfiles(char *input_file, FILE **inp)
{
    if((*inp=fopen(input_file, "r"))==0)
        printf("failure to open files.\n");
    return;
}

/*Close Files*/
void closefiles(FILE *inp)
{
    fclose(inp);
    return;
}

/*File of Data*/
void file_specify(char *filename[])
{
    filename[0]="c:\\degreeday\\1979.txt";
    filename[1]="c:\\degreeday\\1980.txt";
    filename[2]="c:\\degreeday\\1981.txt";
    filename[3]="c:\\degreeday\\1983.txt";
    filename[4]="c:\\degreeday\\1984.txt";
    filename[5]="c:\\degreeday\\1985.txt";
}

```

```

        filename[6]="c:\\degreeday\\1986.txt";
        filename[7]="c:\\degreeday\\1987.txt";
        filename[8]="c:\\degreeday\\1988.txt";
        filename[9]="c:\\degreeday\\1991.txt";
        filename[10]="c:\\degreeday\\1992.txt";
        filename[11]="c:\\degreeday\\1993.txt";
        filename[12]="c:\\degreeday\\1994.txt";
        filename[13]="c:\\degreeday\\1995.txt";
    }

    /*Create Data*/
    void create_degree_data(struct degreeday **dd)
    {
        int i=0;
        (*dd)=(struct degreeday *)malloc(sizeof(DEGREEDAY));
        for(i=0;i<14;i++)
            (*dd)->date[i]=0;
        (*dd)->maxTemp=0;
        (*dd)->minTemp=0;
        (*dd)->numRww=0.0;
    }
    /*Set the Degree to Zero*/
    void degree_data_toZero(struct degreeday **dd)
    {
        int i=0;
        for(i=0;i<14;i++)
            (*dd)->date[i]=0;
        (*dd)->maxTemp=0;
        (*dd)->minTemp=0;
        (*dd)->numRww=0.0;
    }
    /*Create the Data Structure of Result*/
    void create_result(struct result **resu)
    {
        int i=0;
        (*resu)=(struct result *)malloc(sizeof(RERESULT));
        (*resu)->threshold=0;
        (*resu)->rwwNumber=0.0;
        (*resu)->startDay=0;
        (*resu)->avgDDF=0.0;
        for(i=0;i<14;i++)
        {
            (*resu)->DDF[i]=0.0;
            (*resu)->estDDFDay[i]=0;
            (*resu)->actDDFDay[i]=0;
        }
    }
}

/*Create the Data Structure of RMSE*/
void create_meanError(struct meanerror **meanerr)
{
    (*meanerr)=(struct meanerror *)malloc(sizeof(MEANERROR));
    (*meanerr)->rmseF=0.0;
    (*meanerr)->meanF=0.0;
}

/*Create the Data Structure of Years*/
void create_year(char *year[])
{
    year[0]="1979";
    year[1]="1980";
    year[2]="1981";
    year[3]="1983";
    year[4]="1984";
    year[5]="1985";
    year[6]="1986";
    year[7]="1987";
    year[8]="1988";
    year[9]="1991";
    year[10]="1992";
}

```

```

        year[11]="1993";
        year[12]="1994";
        year[13]="1995";
    }

/* Read-Data Module
Input: parameters (temperature threshold, number of weevils caught,
      start dates for accumulating heat unit)
Output:parameters (total Degree*Day)
Process:calculate the degree*day for each year
*/
void read_data(RESULTPTR *result,int heatF,double rwwNumber,int startDay)
{
    int i=0,j=0;
    double DD=0.0;
    double totalRww=0.0;
    FILE *inp=NULL;
    char *input_file[15]={NULL};
    DDPTR ddptr=NULL;

    create_result(result);

    (*result)->threshold=heatF;
    (*result)->rwwNumber=rwwNumber;
    (*result)->startDay=startDay;

    file_specify(input_file);

    while(input_file[i]!=NULL && i<14)
    {
        openfiles(input_file[i],&inp);
        create_degree_data(&ddptr);
        j=0;
        while(!feof(inp) && totalRww<=rwwNumber)
        {
            j++;
            fscanf(inp,"%s%lf%d%d",ddptr->date,&ddptr->numRww,
                &ddptr->maxTemp,&ddptr->minTemp);

            totalRww=totalRww+ddptr->numRww;
            (*result)->actDDFDay[i]++;

            if(j>=startDay)
            {
                single_sine_lowInter(ddptr,&DD,heatF);
                degree_day_DDF(DD,&(*result)->DDF[i]);
            }
            DD=0.0;
        }
        closefiles(inp);
        i++;
        totalRww=0.0;
    }
}

/*Output Result*/
void print_result(RESULTPTR minErrF,MEANERRORPTR meanErr,RESULTPTR predict)
{
    int i=0;
    double avgDDF=0.0;
    int errDDF=0;

    FILE *outp=NULL;
    char *output_file="c:\\degreeday\\result1_10_50_60_30_60.txt";
    char *year[15]={NULL};

    create_year(year);

    outp=fopen(output_file,"wt");

```

```

        fprintf(outp,"==== degree day model output =====\n\n");
        fprintf(outp,"Minmium RMSE for predicting flight is at cumulative total
weevils=%.2lf\n",
            minErrF->rwwNumber);
        fprintf(outp,"Minmium RMSE for predicting flight is at the temperature
threshold=%dF\n",
            minErrF->threshold);
        fprintf(outp,"Minmium RMSE for predicting flight is at start day=%dDay\n\n",
            minErrF->startDay);

        fprintf(outp,"DDF--degree days of 14 years\n\n");

        for(i=0;i<14;i++)
        {
            fprintf(outp,"year=%s\tDDF=%.2lf\n\n",year[i],minErrF->DDF[i]);
        }

        fprintf(outp,"the averages of degree days are:\n");
        fprintf(outp,"average DDF=%.2lf \n",minErrF->avgDDF);
        fprintf(outp,"the predictions of 14 years are:\n\n");
        fprintf(outp,"year//estimated days//actual days//difference:\n\n");

        for(i=0;i<14;i++)
        {
            errDDF=minErrF->estDDFDay[i]-minErrF->actDDFDay[i];

            fprintf(outp,"%s\t\t%d\t%d\t%d\n",year[i],
                minErrF->estDDFDay[i],minErrF->actDDFDay[i],
                errDDF);

        }
        i=0;
        fprintf(outp,"mean and root mean square error are:\n");
        fprintf(outp,"mean\tRMSE\n");
        fprintf(outp,"%.2lf\t%.2lf\n",meanErr->meanF,meanErr->rmseF);
        fprintf(outp,"\n");
        fprintf(outp,"prediction of 2001//estimated days//actual days\n\n");

        year[0]="2001UvLight";
        year[1]="2002UvLight";
        year[2]="2003UvLight";

        for(i=0;i<5;i++)
        {
            if(predict!=NULL)
                fprintf(outp,"%s\t%d\t%d\n",year[i],
                    predict->estDDFDay[i],predict->actDDFDay[i]);
        }
    }

/*Sine Wave Method to Calculate the Degree-Day*/
void average_DD(double DDF[],double *avgDDF,int replicate)
{
    int i=0;
    double totalDDF=0.0;

    for(i=0;i<replicate;i++)
    {
        totalDDF=totalDDF+DDF[i];
    }
    *avgDDF=totalDDF/replicate;
}

void degree_day_DDF(double DD,double *DDF)
{
    *DDF=*DDF+DD;
}

void single_sine_lowInter(struct degreeday *dd,double *DD,int heatF)

```

```

{
    double avg=0.0,deD=0.0;

    if(dd->maxTemp<=heatF)
        *DD=0.0;
    else if(dd->minTemp<heatF)
        intercept_low_threshold(dd->maxTemp,dd->minTemp,heatF,DD);
    else if(dd->minTemp>=heatF)
        entirely_between_threshold(dd->maxTemp,dd->minTemp,heatF,DD);
    else;
    return;
}

void intercept_low_threshold(int maxTemp,int minTemp,int heatF,double *DD)
{
    double avg=0.0,deD=0.0;

    avg=(maxTemp-minTemp)/2;
    deD=(heatF-(maxTemp+minTemp)/2)/avg;
    deD=asin(deD);

    *DD=(1/3.1415926)*(((maxTemp+minTemp)/2-heatF)*(3.1415926/2-deD)
        +avg*cos(deD));
    return;
}

void entirely_between_threshold(int maxTemp,int minTemp,int heatF,double *DD)
{
    double avg=0.0;

    avg=(maxTemp+minTemp)/2;

    *DD=avg-heatF;

    return;
}

/* Read_Data_Verify Module
Input: Degree-Day
Output:parameter (days for each year to reach the averaged degree*day)
Process:calculate the degree*day for each year
*/

void read_data_verify(RESULTPTR *result,int heatF,double rwwNumber,int startDay)
{
    int i=0,j=0;
    double DD=0.0;

    FILE *inp=NULL;
    char *input_file[15]={NULL};
    DDPTR ddptr=NULL;
    RESULTPTR newresult=NULL;

    average_DD((*result)->DDF,&(*result)->avgDDF,14);

    create_result(&newresult);

    file_specify(input_file);

    while(input_file[i]!=NULL && i<14)
    {
        openfiles(input_file[i],&inp);
        create_degree_data(&ddptr);
        j=0;
        while(!feof(inp) && newresult->DDF[i]<=(*result)->avgDDF)
        {
            j++;
            fscanf(inp,"%s%lf%d%d",ddptr->date,&ddptr->numRww,
                &ddptr->maxTemp,&ddptr->minTemp);
            (*result)->estDDFDay[i]++;
        }
    }
}

```

```

        if(j>=startDay)
        {
            single_sine_lowInter(ddptr,&DD,heatF);

            if(newresult->DDF[i]<=( *result)->avgDDF)
            {
                degree_day_DDF(DD,&newresult->DDF[i]);
            }
        }
        DD=0.0;
    }
    closefiles(inp);
    i++;
}

void get_mean_error(MEANERRORPTR *meanErr,RESULTPTR result,int replicate)
{
    int i=0;
    double totalF=0.0,totalSquareF=0.0;
    double differ=0.0;
    for(i=0;i<replicate;i++)
    {
        differ=(result->actDDFDay[i]-result->estDDFDay[i]);
        if(differ<0)
            differ=-differ;
        totalF=totalF+differ;
        totalSquareF=totalSquareF+pow(differ,2);
    }
    (*meanErr)->meanF=totalF/replicate;
    (*meanErr)->rmseF=sqrt((totalSquareF/replicate));
}

/*  Get_Minimize_Error Module
Input: parameter (days from actual days and predicted days)
Output:parameter (minimum RMSE)
Process:calculate minimum RMSE
*/

void get_minimize_error(RESULTPTR *minErrF,RESULTPTR result,
                       MEANERRORPTR *meanErr,FILE *outp)
{
    MEANERRORPTR newMeanErr=NULL;

    create_meanError(&newMeanErr);

    get_mean_error(&newMeanErr,result,14);

    fprintf(outp,"%0.2f %d %d %0.2f\n",result->rwwNumber,
        result->threshold,result->startDay,newMeanErr->rmseF);

    if((*meanErr)->rmseF==0.0 || (*meanErr)->rmseF>newMeanErr->rmseF)
    {
        (*meanErr)=newMeanErr;
        (*minErrF)=result;
    }
}

/*  Predict_Event Module
Input: parameter (degree*day with minimum RMSE)
Output:parameter (difference in days)
Process:calculate the difference between actual and predicted days
        for the recent years
*/
void predict_event(RESULTPTR result,RESULTPTR *predict)

```

```

{
    int i=0,j=0;
    double DD=0.0;

    FILE *inp=NULL;
    char *input_file[4]={NULL};
    double totalRww=0.0;
    DDPTR ddptr=NULL;

    input_file[0]="c:\\degreeday\\2001uv.txt";
    input_file[1]="c:\\degreeday\\2002uv.txt";
    input_file[2]="c:\\degreeday\\2003uv.txt";

    create_result(predict);
    create_degree_data(&ddptr);

    while(input_file[i]!=NULL && i<3)
    {
        openfiles(input_file[i],&inp);

        j=0;
        while(!feof(inp) && ((*predict)->DDF[i]<=result->avgDDF
            || totalRww<=result->rwwNumber))
        {
            j++;
            fscanf(inp,"%s%lf%d%d",ddptr->date,&ddptr->numRww,
                &ddptr->maxTemp,&ddptr->minTemp);
            totalRww=totalRww+ddptr->numRww;

            if(j>=result->startDay)
            {

                single_sine_lowInter(ddptr,&DD,result->threshold);

                if(totalRww<=result->rwwNumber)
                {
                    (*predict)->actDDFDay[i]++;
                }
                if((*predict)->DDF[i]<=result->avgDDF)
                {
                    degree_day_DDF(DD,&(*predict)->DDF[i]);
                    (*predict)->estDDFDay[i]++;
                }
                else;
            }
            DD=0.0;
            degree_data_toZero(&ddptr);
        }
        totalRww=0.0;
        (*predict)->actDDFDay[i]=(*predict)->actDDFDay[i]+result->startDay;
        (*predict)->estDDFDay[i]=(*predict)->estDDFDay[i]+result->startDay;
        closefiles(inp);
        i++;
    }
}

```

## VITA

Li Zou, the first son of Yingbiao Zou and Ruixiu Chen, was born in Gaoan, Jiangxi Province, on March 10, 1973. He obtained his bachelor of science degree in chemistry from Kunming Institute of Technology in 1995. He successfully obtained his master of science in zoology from Kunming Institute of Zoology, The Chinese Academy of Sciences, in 1998. Li was accepted as a doctoral student in the Department of Entomology in the spring, 2000 at the Louisiana State University and Agricultural and Mechanical College. His research was conducted under Dr. Michael J. Stout. After graduation, Li will continue his career in the area of natural resource management.