July 1, 1993

LCMR Final Status Report - Summary - Research
LCMR WORK PROGRAM

I. Insecticide Impact on Wetland and Upland Wildlife

Program Manager: Alfred H. Berner
Farmland Wildlife Populations and Research
Department of Natural Resources
Rte 1, Box 181
Madelia, MN 56062
507-642-8478

A. M.L. 91 Ch. 254 Art. I Sec.14 Subd: 9(a) Appropriation: $650,000
Balance: $0

Insecticide Impact on Wetland and Upland Wildlife: This appropriation is from the Minnesota (MN) Environment and Natural Resources Trust Fund to the Commissioner of Natural Resources to research the effect of insecticides on wetland and upland wildlife and habitats.

II. NARRATIVE: State-mandated control of grasshopper outbreaks as well as routine agricultural chemical applications have resulted in spraying of broad-spectrum insecticides on non-cropped wetland and upland acres [e.g., roadsides, Conservation Reserve Program (CRP) and Reinvest in MN (RIM)]. Habitat components provided by these acres are critical for the survival of many waterfowl, songbirds, and upland game bird species. Recent studies conducted by Patuxent Wildlife Research Center (PWRC) biologists in North Dakota (ND) documented direct mortality from aerially applied ethyl parathion to waterfowl ducklings and to the aquatic invertebrates that comprise an important component of female waterfowl and duckling's diets. Information is needed to determine how the growth and survival of ducklings that do not die from the insecticide application is affected when their prey base is depleted by insecticide drift or over-spray. Some insecticides have also been shown to cause direct mortality on upland birds while others have affected populations by reducing or eliminating insects and other invertebrates that comprise the primary food source of young birds. By increasing our understanding of the effects of agricultural insecticides on the quality of prairie wetland and upland habitats, information will be generated to assist wetland managers, farmers, agriculture extension personnel, and state and federal agencies in providing quality prairie wetland and upland habitat while continuing to meet the needs of agriculture. These 2 complimentary and coordinated study components are designed to determine the extent of direct and indirect insecticide impacts on upland and wetland birds and their invertebrate food base.

A. FINAL ABSTRACT: From 1991 through 1992, we conducted a series of field experiments designed to determine the direct and indirect effects of application of agricultural insecticides on waterfowl and upland game birds.

Wetland Research: In 1991, 1 mallard brood was allowed to forage on 4 wetlands located on U. S. Fish and Wildlife Service (USFWS) Waterfowl Production Areas (WPA's) in western MN. Wetlands were paired on the basis of size (large or small), and 1 wetland in each pair was treated aerially with Asana® XL at the maximum label rate for grasshopper control on non-crop lands. The mallard brood was allowed to forage on 2 pairs of wetlands (1 large pair and 1 small pair) over a 2-day period. Invertebrate populations were depressed in treated wetlands for up to 15 days after insecticide application and amphipod abundance was reduced to 0. Ducklings were observed foraging on dead invertebrates, and initially gained mass faster on treated than on untreated wetlands. However, this trend was reversed over the course of the experiment until rate of mass gain was similar on treated and untreated wetlands.

In 1992, 12 imprinted mallard broods were allowed to forage exclusively on either treated or untreated wetlands located on USFWS WPA's in western MN. We observed no significant effect of application of Asana® XL on duckling mass 15 days after treatment, although mean survival for broods reared on treated sites ($\bar{x}$ = 40.6%, n = 6) was significantly lower than for broods reared on untreated sites ($\bar{x}$ = 65.6%, n = 5) (t = 3.83, 8 df, P = 0.005). Morphological development did not differ between birds allowed to forage on treated versus untreated wetlands.

Upland Research: In 1991, direct effects on upland game birds were investigated by exposing 2-week old, 6-week old, and ≥ 14-week old ring-necked pheasants *(Phasianus colchicus)* and gray partridge *(Perdix perdix)* to a single field application of Asana® XL, Malathion, or Furadan. In both years of the study, indirect effects of insecticides were measured by monitoring direct effects of insecticide application on invertebrate populations important in the diets of upland game bird chicks. In addition to monitoring invertebrate populations, we used imprinted broods of ring-necked pheasants (1992) to assess potential indirect effects of Asana® XL application on invertebrate-dependent juvenile birds.

In all experiments, invertebrate abundance and biomass were reduced following application of insecticides. In 1991, pheasant and partridge exposed to direct application of Asana® XL, Malathion, or Furadan did not exhibit any obvious signs of acute toxicity to insecticides and brain cholinesterase (ChE) activity was not depressed in these birds.

In conjunction with the wetland portion of the study in 1992, 2 broods of imprinted ring-necked pheasants were allowed to forage exclusively on either treated or untreated upland

areas located on USFWS WPA's, also in western MN. There was no effect of application of Asana® XL on daily mass change of pheasant chicks, even though both invertebrate number and biomass were reduced on treated plots following insecticide application. Feeding rates of pheasant chicks were not good indicators of growth rates and chicks were observed foraging on dead invertebrates following insecticide application.

Summary: We observed no direct acute effects of field application of Asana® XL on mallard ducklings nor of Asana® XL, Malathion, or Furadan on ring-necked pheasants or gray partridge. Invertebrate populations were significantly reduced following insecticide application in all field experiments, suggesting the potential for indirect effects on ducklings and game bird chicks. Both mallard ducklings and pheasant chicks foraged on invertebrates killed by insecticides, although daily mass changes were not different between birds that foraged on treated versus untreated areas in 1992. In the case of mallards, brood survival was reduced as a result of aerial application of Asana® XL and mediated by direct impacts on invertebrate populations, suggesting that subtle effects of insecticide application on food availability may result in decreased survival and recruitment.

III. OBJECTIVES:

A. Determine the extent of direct and indirect mortality on young mallards, pheasants, and gray partridge caused by approved insecticides used to control insects (e.g., grasshoppers).

A.1.1. Narrative (wetlands): Determine the extent of direct and indirect mortality in imprinted mallards and their associated wetland invertebrate food base from Asana ® XL used at recommended rates to control crop insect pests.

The wetland portion of the project will focus on mallard ducklings and aquatic invertebrates.

A.1.2. Narrative (uplands): Determine the extent of direct and indirect mortality on pen-enclosed ring-necked pheasants and gray partridge and their associated invertebrate food base from approved insecticides used to control insect crop pests.

The upland portion of the project will focus on gray partridge, pheasants, and terrestrial invertebrates.

A.2.1. Procedures (wetlands):

A.2.1.1. Protocol Development: Utilizing available information generated by the USFWS, PWRC and information available in the literature, details of the research protocol were completed. This research was conducted under faculty supervision by 1 graduate degree student in Wildlife Conservation at the University of Minnesota (U of MN) and 1 staff scientist; these people were responsible for coordinating the majority of the field work.

A.2.1.2. Study Site: The field portion of this study began April 1991 and was concluded August 1992. In the first field season, we selected 4 wetlands on federal WPA's of similar type, size, biotic character, and surrounding land-use pattern. Wetlands were paired on the basis of proximity, size, and type. One wetland in each pair served as a reference while the second served as a treated wetland. In 1992, we selected 12 study wetlands following the approach used in the previous year. The study was designed to monitor the quality of the wetland ecosystem as waterfowl brood-rearing habitat. Not only did we measure different aspects of the wetland to determine the impact insecticides may have had on the ecology of this ecosystem, but, we also studied the growth and behavior of broods released onto the wetlands to demonstrate the potential sub-lethal impacts insecticides might have on waterfowl.

A.2.1.3. Insecticides: Asana® XL, a synthetic pyrethroid insecticide that is highly toxic to invertebrates (Coats et al. 1989, U.S. Environmental Protection Agency 1989) but is essentially non-toxic to birds and mammals, was applied by a licensed aerial applicator. The deposit of insecticide in the wetland was measured using 3 circular filter papers (spray deposit cards) on stakes located at each of the invertebrate sampling stations. This method is identical to the methods used previously in ND and MN pesticide studies.

A.2.1.4. Experimental Design:

Impact of insecticide spraying on selected aquatic invertebrate populations: Three transects were established in each wetland. Each transect began in the center of the wetland and radiated at an angle of 120° from other transects. Two permanent invertebrate sampling stations were established along each transect at the open water/deep marsh zone interface, and in the shallow marsh zone.

Invertebrates important in the diet of young ducklings were sampled in 1991 and 1992. In 1992, we sampled aerial insects that occurred in the area accessible to foraging ducklings using emergence traps. An emergence trap consists of a floating frame from which a mesh tent is suspended. Aquatic insects emerging into this trap are funneled toward the top of the tent and trapped in a collection jar filled with a preservative. We identified each invertebrate (at least to Family) and weighed taxonomic groups to obtain an estimate of the types of invertebrates and biomass available to ducklings foraging at the water surface. Six emergence traps were placed on each wetland. The traps were set in the wetlands 1 day prior to, and collected 1

day subsequent to spray, and then set and collected at weekly intervals until 28 days post-spray. Invertebrate populations occurring in the water column were sampled during the season with activity traps (6 traps/wetland) and 5 cm diameter core samplers (3 cores/wetland). Aquatic invertebrates were sampled 1 day pre-spray, 1 day post-spray, at 3-day intervals for the first 15 days post-spray, and at weekly intervals from 15 to 28 days post-spray. We compared numbers, species, and biomass of invertebrates collected during the same time intervals in treated and reference wetlands and compared differences using 1-way analysis of variance (ANOVA) and 2 sample t-tests.

In addition, the effect of Asana® XL on invertebrates was also evaluated using in-situ field bioassays during 1991 and 1992. This involved placing cages containing known numbers of clean, same age, laboratory cultured amphipods and chironomid larvae in each study wetland 1 hour before application of the insecticide. Cages were then recovered within 4 hours after spray and the survival of the organisms in them was determined.

Growth and behavior of game farm mallards released onto study wetlands: This portion of the study was conducted in 1992, while a pilot study was conducted in 1991. The effect of Asana® XL on waterfowl was evaluated using mallard ducklings imprinted on human observers. Imprinted ducklings have been used previously to assess impacts of low pH (Hunter et al. 1986) and insecticides (Hunter et al. 1984, Cooper et al. 1989) on wetland wildlife. Ducklings were allowed to forage on study wetlands from 1 to 2 days before the insecticide was applied to treated wetlands. Each duckling was individually marked with colored flagging tape and numbered web tags, weighed daily, and measures of wing, culmen, and tarsus were recorded at 3-day intervals.

Large predator-proof fences (Lokemoen et al. 1982) were not used to enclose wetlands as these are designed to enhance natural waterfowl nest success on upland sites. These large fences have been found to be ineffective in predator-proofing wetlands. Any mink *(Mustela vison)* that are able to pass the barricade can establish a den in the wetland area and prey upon the eggs of hens that nest within the enclosure (J. T. Lokemoen USFWS, Northern Prairie Research Center, pers. comm.). Our study was designed to determine the impact of pesticide-induced perturbations on aquatic invertebrates and on the subsequent growth and survival of ducklings. For our study purposes, ducklings imprinted on human observers were allowed to forage on the wetlands under the surveillance of human observers. This approach facilitated daily weighing, while the presence of the observer on the wetland (acting as a hen) deterred predation. Reliance on natural reproduction is too risky and does not allow for daily weight gain/loss determinations.

Behavior of broods on treated and reference wetlands was monitored using scan sampling techniques. The behavior of individual ducklings (i.e., rest/sleep, searching for food, foraging above or below water surface) was recorded with the use of laptop computers. In addition, brood location (i.e. emergent vegetation, open water, or upland) on each study wetland was noted. We were primarily interested in gross differences in the ability of ducklings to forage efficiently, leading to potential impacts on growth rate differences between broods reared on treated and reference wetlands due to changes in the invertebrate forage base as a result of insecticide introduction into the wetland. These differences were tested using 2 sample t-tests. Differences in weight and morphometry were compared between birds reared on treated and reference wetlands with 2 sample t-tests.

Laboratory Toxicity Tests: In order to completely assess the toxicity of Asana® XL to aquatic invertebrates, controlled laboratory studies were conducted using 2 laboratory cultured species, both of which are commonly found in wetlands and important food items for ducklings. They were exposed to the same formulation and concentration of Asana® XL as applied under field circumstances. By controlling temperature, light source, food base, and dissolved oxygen, the biological impact of the chemical can be ascertained when all other conditions are optimized and held constant. Mortality was evaluated in flow-through toxicity tests. Screening of field collected sediment pore water (a reservoir for many aquatic contaminants) was also assessed using MICROTOX®, a bioluminescing bacteria that reacts to toxic concentrations of contaminants in water. This laboratory work confirmed mortality patterns seen in field results.

A.2.2. Procedures (uplands):

A.2.2.1. Protocol Development: Initially, a comprehensive literature review was conducted and details of the research protocol were finalized based on published literature and study site restrictions and logistics. This research was conducted under faculty supervision by 1 Master of Science degree student in Wildlife Conservation at the U of MN, and the student was responsible for coordinating the majority of the field work.

A.2.2.2. Study Site: Field research in 1991 was conducted at the MN Department of Natural Resource's (DNR) Farmland Wildlife Populations and Research (FWPR) facility near Madelia, MN. A 20-acre upland grass-legume field was manipulated to provide habitat plots that were used in a field experiment. Habitat plots in 1991 were similar in vegetative cover to roadsides, CRP, and RIM habitat and were approximately 16 m wide and 25 m long. Ten blocks of 5 plots each were created by mowing buffer zones of 8 m between adjacent plots and between plots and nearby habitat. Upland habitat dominated by cool-season grasses and legumes is important

brood habitat for both pheasants and gray partridge in south-central MN (Nelson et al. 1990) and is similar to roadside habitats that comprise a significant proportion of non-agricultural habitats in the region (Joselyn and Tate 1972, Varland 1985).

Field research in 1992 was conducted on upland areas up to 2 ha in size on USFWS WPA's associated with wetlands in western MN. These areas had been seeded previously with warm-season native grasses but cool-season grasses and legumes were also present.

A.2.2.3. Insecticides: In 1991, we selected 3 broad-spectrum insecticides that vary in toxicity to birds (as measured by the lethal dose to 50% of test organisms, expressed as an LD_{50}) reported in the literature] and that are commonly applied to agricultural land in MN. The 3 categories of insecticides, based on toxicity were: LD_{50} < 20 mg active ingredient (ai)/ kg body weight (Furadan), LD_{50} between 100 and 300 mg ai/ kg body weight (Malathion), and LD_{50} > 2000 mg ai/ kg body weight (Asana® XL). All pesticides selected were water soluble to minimize possible confounding effects of carriers, and are registered for use on agricultural land and crops commonly grown in MN.

In 1991, insecticides were applied from the ground with a hydraulic boom-sprayer pulled behind a vehicle. A plot width of 16 m allowed each plot to be sprayed with 2 passes of the sprayer. Insecticides were applied at label rates and application rates were determined by mixing known amounts of insecticides with water in the application tank and by analyzing spray cards placed in habitat plots prior to spraying. Drift from one plot to another was minimized by applying the insecticides when environmental conditions were optimal for spraying (e.g., no wind).

In 1992, the broad-spectrum insecticide Asana® XL was sprayed on the study areas in conjunction with the wetland portion of the study. This insecticide is registered for use on cropland and is commonly used on agricultural areas in MN. Asana® XL was aerially applied at a rate recommended for grasshopper control in MN.

A.2.2.4. Experimental Design: In 1991, insecticides were applied twice during the course of the experiment to approximate standard application practices in the region, and to evaluate both the direct and indirect effects of the insecticides. The 10 habitat blocks were divided into 2 groups of 5 blocks. One group of 5 blocks was sprayed in mid-June and both groups were sprayed in mid-July. Within each block, application of each insecticide, application of a water control, plus an untreated control plot, were assigned randomly, for a total of 5 plots per block, and 5 replications within each block. An additional plot was added to each replicate block to reduce the probability that plots randomly assigned to the same treatment would be adjacent to one another.

In mid-June 1991, 1 group of replicate blocks was sprayed and insect populations were monitored by sweep netting prior and subsequent to spraying at 5-8 day intervals. In mid-July, both groups of blocks were sprayed and 5 pheasants and 5 gray partridge of 3 age classes (2 weeks, 6 weeks, and ≥14 weeks) were placed in each plot in the groups of blocks that were not sprayed in June. Pheasants and gray partridge were directly subjected to spraying. Insect populations were monitored in this group of blocks prior to and after insecticide application.

Indirect effects on game birds were investigated by monitoring direct impacts on insect populations, which comprise the majority of food for chicks through their first 5-6 weeks of life (Loughrey and Stinson 1955, Southwood and Cross 1969, Erpelding et al. 1987). Sweep net samples were collected at 2 sites within each plot (e.g., Nelson et al. 1990) and insects were identified to Order, Family, or Genus, depending upon ease of identification and importance in the diet of pheasants and gray partridge as determined from published literature. Insect samples were oven dried and weighed to determine mass.

Direct effects on game birds were investigated by monitoring behavior and mortality for up to 14 days post-spray. Birds were removed from study plots within 1 hour after insecticide application and monitored in holding pens. Zero to 5 birds per age class per species per treatment were euthanized at 1, 4, 7, 10, and 14 days after spraying. Brain ChE activity was determined from all birds that died subsequent to spraying and from a random ssample of euthanized birds.

In 1992, Asana® XL was applied twice during the course of the experiment. Two upland areas were treated in mid-June and 2 were treated in mid-July. Two broods consisting of 10 ring-necked pheasant chicks were imprinted on human observers and were allowed to feed exclusively on either insecticide-treated or untreated study areas. Birds were monitored up to 15 days post-spray. Two additional groups of 5 ring-necked pheasant chicks fed alternately on the treated and untreated areas.

Indirect impacts of the insecticide on pheasants were measured by comparing differential mass change for each brood and by monitoring feeding rates of the broods. Sweep net samples were collected 4 times daily in the areas in which chicks were actively feeding to correlate with mass change and feeding rates. Insect samples were identified and placed into taxonomic groups important in the diet of young pheasants, and oven dried and weighed to determine biomass.

A.2.2.5. Follow Up Studies: Based on the results of the 1991 experiment, we further evaluated the indirect effects of agricultural insecticides on upland game birds in 1992, as described above. In conjunction with the wetland portion of this project,

we compared the growth and feeding rates of imprinted pheasant broods (Kimmel and Healy 1987) in upland habitats associated with wetlands on both treated and untreated reference areas. Sweep net samples of insects were collected prior to and subsequent to insecticide application. Sweep net samples were also collected in the immediate vicinity of feeding broods.

A.2.3. Results and Discussion (Wetlands)

Insecticide: The average rate of Asana® XL deposition on spray cards in the treated wetlands was slightly greater than the nominal rate due to overlap of overspray passes.

Impact of insecticide spraying on selected aquatic invertebrate populations: In the treated wetlands during 1991, the total number of invertebrates captured in activity traps declined immediately following application of Asana® XL. This decline persisted for approximately 11 days, at which point the recovery of invertebrate abundance had begun. The portion of the invertebrate community sampled in activity traps returned to abundance levels similar to those sampled in reference wetlands within approximately 18 days post-treatment, with the notable exception of amphipods, which were essentially eliminated following treatment. In benthic cores, numbers of chironomid larvae were not reduced by the application of Asana® XL during 1991. Numbers of amphipods in cores were reduced to 0 immediately following treatment. Amphipods were still not present in either activity trap or benthic core samples collected from treated wetland sites 1 year later.

The effect of Asana® XL on cultured invertebrates was also evaluated with in-situ field bioassays. Both amphipods and chironomid larvae in these bioassay cages experienced significant acute mortality due to application of Asana® XL. Additionally, amphipods demonstrated a greater degree of sensitivity to Asana® XL than chironomids. These results confirm the treatment response we observed in the naturally occurring invertebrates from these taxonomic groups when treated with Asana® XL.

In 1992, numbers of invertebrates in activity traps in treated wetlands declined immediately after application of Asana® XL and remained at levels lower than those observed in untreated wetlands for the rest of the 15 day post-treatment time period. Insects captured in emergence traps consisted primarily of chironomids, which occurred in 99% of the samples collected and constituted 91% of the overall mean number of insects in samples. In treated wetlands, the number of emerging insects, including numbers and biomass of chironomids, declined immediately after application of Asana® XL and remained at levels lower than those observed in untreated wetlands throughout the 15 day post-treatment time period.

Growth and behavior of mallard ducklings released onto study wetlands -1991: No duckling mortality was observed due to direct toxicity of Asana® XL. Immediately after application of Asana® XL, duckling mass gain per feeding session was higher on treated wetlands than on reference wetlands. Duckling mass gains that occurred on treated and reference wetlands were nearly equal at 8 days post-treatment, however, birds gained more mass when they foraged on reference compared to treated sites during the 9 to 17 post-spray time period. Finally, by 18 to 20 days after spray, mass gains were nearly equal for forage bouts on both reference and treated wetlands. In the case of a single mallard brood allowed to forage on multiple wetlands, as in this study during 1991, change in mass per feeding session is an indication of short-term food intake as opposed to long-term growth. A summary of behavioral observations indicated that the activity budgets of ducklings on the reference wetlands were consistent throughout the duration of the study. In contrast, the feeding and resting patterns of ducklings on the treated wetlands varied. During the 6 days immediately after application of insecticide, ducklings foraged less and rested more on treated wetlands than they did on reference wetlands. This trend was reversed from 8 to 12 days after Asana ® XL application.

Growth and behavior of mallard ducklings released onto study wetlands - block 1, May 1992: The daily mass changes of birds reared on both reference and treated wetlands were influenced by ambient temperatures recorded during block 1. Daily mean percent mass change per brood was strongly correlated with mean daily temperature (all r's ≥ 0.83, all P's ≤ 0.01). During the first 4 days post-treatment we observed 4 ducklings from a single treated wetland that apparently were unable to maintain sufficient mass to survive. The activities of this brood during this time period were marked by constant distress calling and increased time spent on food searching behaviors, including searching activities in the upland habitat adjacent to the wetland. This brood experienced 100% mortality at 5 days post-spray (25 May). The mean daily mass for all birds increased for 2 days following insecticide application (20 May), followed by 3 days of consistent mass loss for all birds. Surviving broods were sheltered and provided supplemental food from noon on 25 May to noon on 27 May due to inclement weather and were subsequently allowed to forage exclusively on wetlands for the remaining 9 days of this block. The mean duckling weights for all of the surviving broods were not different (reference $\bar{x}$ = 55.9 and 56.0, treatment $\bar{x}$ = 56.1) following the first full day (27 May) that they returned to foraging exclusively on wetlands. However, the mean daily mass of the surviving treatment brood remained consistently lower than the 2 reference broods for the remainder of this block. Mean brood weights (at 15 days post-treatment) for

block 1 were significantly different for birds reared on treated (n = 7, $\bar{x}$ = 66.6 g) versus reference wetlands (n = 13, $\bar{x}$ = 84.7 g) (t = -2.73, 18 df, P = 0.014). Individual duckling survival at 15 days post-treatment for block 1 was significantly higher for birds reared on reference wetlands (52%) than for birds reared on treated sites (25%) (χ^2 = 4.10, 1 df, P = 0.043).

Block 1 experimental design changes: Research was interrupted from noon on 25 May to noon on 27 May due to temperatures that ranged from 6.1 to 10.5 C below normal (G. Spoden MN DNR, Midwestern Climate Center, pers. comm.). Prior to this time period we observed 4 days of consistent mass loss that we felt were weather related and threatened duckling survival. In an effort to preserve the integrity of the experimental design of block 1, we sheltered and provided supplemental food to all surviving birds until the weather improved sufficiently to allow their return to wetlands.

Growth and behavior of mallard ducklings released onto study wetlands - block 2 June 1992: Mean daily masses for 2 broods reared on treated wetlands and 1 brood reared on a reference wetland did not differ throughout the course of block 2 until the final sampling day. Mean brood masses for block 2, at 15 days post-treatment, were significantly different for birds reared on treated (n = 13, $\bar{x}$ = 220.8 g) versus reference wetlands (n = 7, $\bar{x}$ = 182.9 g) [t = 2.35, 18 df, P = 0.031; note that an F-test for equal variances ($F_{6,12}$ = 3.50, P = 0.031) indicated that the variances were not equal; this inequality may result in the appearance of a more powerful t-test than if the variances were equal. Individual duckling survival at 15 days post-treatment for block 2 was significantly higher for birds reared on the reference wetland (100%) than the 2 treated sites (56%) (χ^2 = 6.24, 1 df, P = 0.012). Nine birds were lost and never recovered. These losses are likely attributable to mink depredation. Observations of gut contents obtained from birds during block 2 revealed that all birds in this block were consuming large quantities of vegetative matter, primarily seeds, at a much earlier age than has previously been reported in the literature (Lees and Street 1977, Perret 1962, Chura 1961).

Block 2 experimental design changes: Cold weather curtailed the morning forage bout for all of the broods on 20 June, until a minimum 10 C air temperature was reached. A brood of birds originally included in block 2 and assigned to a reference site failed to maintain sufficient body mass to insure survival. This condition may have led to the unusually high mortality experienced by this brood through the first 4 days of this block. As a result, all broods in block 2 received supplemental food for 3 evenings during the time when the birds were not on the wetlands. The brood with lower body mass was provided an additional 2 days of supplemental food when they were off the wetland, at which point we removed these birds from this reference site and relocated them to a reference site used in 1991 for the remaining 11 days in block 2. This brood appeared to recover once they were relocated, which may indicate that the original wetland was poor brood-rearing habitat, though the results of invertebrate sampling at this site were inconclusive. This action effectively removed this brood from our study design.

Growth and behavior of mallard ducklings released onto study wetlands-block 3, July 1992: Mean daily masses for broods were nearly equal through 6 days post-treatment (reference $\bar{x}$ = 61.9 and 74.8 g, treatment $\bar{x}$ = 72.3 and 59.4 g), at which point 1 brood on a treated wetland remained consistently lighter than all other broods. This difference was significant when tested at post-spray day 15 (t = -5.09, 22 df, P = 0.00004). Mortality of 4 birds occurred in this brood. These birds all lost mass prior to their death and 3 carcasses were recovered with no apparent signs of injury. We believe that their deaths were attributable to the effect of the insecticide application. Mean brood masses for block 3 at 15 days post-treatment were significantly different for birds reared on treated (n = 11, $\bar{x}$ = 117.7 g) versus reference wetlands (n = 13, $\bar{x}$ = 176.3 g) (t = -3.58, 22 df, P = 0.0017). Duckling survival at 15 days post-treatment for block 3 was higher for birds reared on the reference wetland (54%) than for birds reared on the 2 treated sites (36%) (χ^2 = 1.94, 1 df, P = 0.16). In block 3, 34 birds were lost and never found and 3 decapitated carcasses were recovered. These losses are likely attributable to mink depredation. Observations of gut contents obtained from 1 bird from each of the treated wetlands on the final day of block 3 revealed that these individuals had been feeding on northern leopard frogs (*Rana pipens*).

Block 3 experimental design changes: There were no experimental design changes during block 3.

Results summary-1992:
Morphological measurements of wings and tarsi from ducklings in broods from blocks 2 and 3 were not significantly different between birds reared on reference or treated study sites. The combined mean daily masses for all birds from all 3 blocks were nearly equal throughout the 15 day post-spray period, with the exception of post-spray day 5. Birds reared on reference wetlands were lighter than birds reared on treated study sites. This difference was likely influenced by block 1 reference birds, which survived severe weather but at a reduced body mass. However, birds on reference sites were, on average, heavier than those reared on treated sites at post-spray day 15. This difference, though slight, is important biologically as mass is known be an important factor in determining a bird's ability to thermoregulate and potentially may influence survival.

The trend of duckling survival was similar for all birds, in every block, both treated and reference from -1 to 5 days post-treatment. However from 5 to 15 days post-treatment, we observed consistently lower survival for broods reared on treated sites. The mean percent survival for all broods on post-spray day 15 reared on reference wetland sites ($\bar{x}$ = 65.6%, n = 5) was significantly different from mean percent survival for broods reared on treated study sites ($\bar{x}$ = 40.6%, n = 6) (t = 3.83, 8 df, P = 0.0046).

Difficulties that arose throughout the course of this study and which resulted in supplemental feeding, likely decreased the power of statistical analyses employed to detect differences in morphology, mass, and survival. However, despite the difficulties encountered, statistically significant differences in duckling survival were detected.

A.2.4. Results and Discussion (Uplands)

Insecticides: In 1991, 1 tank mix sample for each insecticide was collected before each spray application, and 1 retain sample was collected from each of the insecticide containers after mixing. Circular filter papers (spray deposition cards) were placed on plots to be sprayed with insecticide and collected after they dried. Chemical analyses confirm that the active ingredients from the insecticides were present in all 3 matrices. Circular filter papers were again used in 1992 to verify deposition of the active ingredient on the treated sites. Chemical analyses of these spray deposition cards also documented deposition of the active ingredient at all treated sites.

Direct Impacts on Birds: In 1991, birds were placed on plots in wire cages, sprayed on 15 and 16 July, and subsequently placed in holding pens until death or until they were euthanized (up to 14 days post-exposure to insecticides). Following spraying, birds were observed in holding pens for obvious behavioral signs of exposure to carbamate, organophosphate, or pyrethroid insecticides (e.g., dysfunction of motor ability). Ring-necked pheasants and gray partridge exposed to insecticides did not exhibit any obvious behavioral abnormalities that can occur with exposure to pesticides. Additionally, we attributed the few deaths that did occur after spraying to injury, heat stress, and/or illness. Heads of all birds were frozen and brain tissue was analyzed from a random sample to determine brain cholinesterase activity.

Effects of Insecticides on Insects (Indirect Effects): In 1991, insects were collected with sticky and pitfall traps until 6 August, while sweep netting continued until 27 August. Insects on sticky traps were counted in the field and identified to Order. Insects collected by sweep netting were identified to Family and/or Order, and placed in size classes within these groups, depending on the ease of identification and the relative importance in the diet of upland game bird young. Pitfall traps were not analyzed because the insects collected in these traps did not make up a significant portion of the diet of upland game bird young.

Invertebrates captured on sticky traps did not appear to be affected by insecticide applications for the 1991 field season. For analysis, the 3 insecticide treatments (Malathion, Furadan, and Asana® XL) were grouped together, as were the 2 control treatments (water and untreated). Lack of a treatment effect may have occurred because the type of insect (small and mobile) that sticky traps were able to measure on our 1991 study site can rapidly recolonize treated areas.

Invertebrates from sweep net samples were identified to Family and/or Order, oven dried, and weighed to determine biomass. For analysis, the 3 insecticide treatments (Malathion, Furadan, and Asana® XL) were grouped together, as were the 2 control treatments (water and untreated). The average number of invertebrates per sweep sample appeared to be impacted by insecticide application. Invertebrate dry mass also appeared to have been impacted by insecticide application. Areas receiving multiple insecticide treatments may show even greater invertebrate biomass reductions when compared to areas receiving only a single treatment.

In 1992, sweep net samples were collected each day at sites that were used by imprinted birds. Invertebrates in these samples were identified to Family and/or Order, and were dried prior to determining mass. Invertebrates (total numbers per sweep net sample) on both study sites appeared to have been reduced by the insecticide application. Invertebrate dry mass on both sites also appeared to have been impacted after insecticide treatment.

Although both invertebrate numbers and dry mass were reduced on both study sites, ring-necked pheasant chicks did not appear to respond to these changes. Birds feeding on the treated area on site 1 generally had a lower average mass change than those birds feeding on the untreated area on site 1, and was observed both before and after insecticide application. However, this relationship was reversed for birds feeding on site 2: the birds feeding on the treated areas generally had a greater average mass change compared to birds feeding on the untreated area.

Feeding rates of the pheasant chicks on site 1 were greater for those birds feeding on the reference area than on the treated area. Those birds feeding on the reference area also gained more mass than the birds feeding on the treated area. However, birds feeding on the treated area of site 2 gained more mass than birds feeding on the reference area, even though there was little difference in their feeding rates.

We believe there are 2 possible explanations for the apparent lack of response. First, initial differences in the invertebrate populations between sites, and between plots

within sites, may have continued after the insecticide application. A minimum "threshold" in invertebrate numbers and/or biomass necessary to result in a measurable effect in the pheasant chicks may not have been reached.

The second explanation is that recolonization of the treated plots by mobile invertebrates occurred rapidly, even though the insecticide kept the invertebrate populations depressed. In this study, ring-necked pheasant chicks were observed consuming dead invertebrates, and this could account for the apparent lack of response of the birds to the changes in living invertebrate abundance caused by the insecticide application.

In this study, insecticide applications to habitat utilized by upland game bird chicks had no significant effect on growth of imprinted pheasant chicks. Our 1992 study consisted of 1 application of Asana® XL at 34 g ai/ha on upland habitat areas approximately 2 ha in size surrounded by similar, untreated habitat. However, the results obtained in this study should be extended to other situations with caution. Multiple insecticide applications, treated areas that are significantly larger than those in this study, or insecticide applications to habitats that may not be recolonized as rapidly as those in this study (e.g., roadsides adjacent to row crops) may make significant indirect effects of insecticide applications on insect-dependent birds more likely.

A.3. Benefits (wetlands and uplands): Initial studies of insecticide effects suggest a high potential to reduce foods of breeding birds and their young or to cause direct mortality of birds. Understanding effects of these chemicals will help develop techniques to minimize negative impacts on wildlife. By incorporating information on insecticide effects, this research will improve the implementation of existing wildlife management plans while aiding farmers in planning frequency of application, chemical selection, and method of application.

IV. EVALUATION:

A. Wetlands: Recent studies completed in ND (Grue et al. 1989) documented direct and indirect mortality on waterfowl and invertebrates from spraying agricultural insecticides on wetlands. The threat of grasshopper outbreaks, as well as tent caterpillars, indicates the necessity of evaluating the potential of a similar problem in MN. The results of this project were evaluated by 1) determining if direct and indirect effects on waterfowl and their food base occurs; 2) determining the extent of chemical contamination of a wetland through routine chemical application; 3) assessing procedural modifications in operations that could suggest the minimization of impacts of agricultural pest control agents on wetland wildlife while maximizing pest control for crop harvest maximization.

B. Uplands: For the FY92-93 biennium this program was evaluated by its ability to 1) assess the direct impact of selected agricultural insecticides on upland game birds; 2) assess indirect effects of selected agricultural insecticides on upland game birds through impacts on invertebrate foods; 3) provide sound scientific data to resource manager, legislators, and producers concerning application of agricultural insecticides in MN.

In the long term, evaluation of this project's success will be in the development of procedures and practices for prescribing and applying agricultural chemicals that minimize potential negative environmental impacts. Concern for the environment as a whole needs to be incorporated into agricultural production practices based on the best available information.

V. QUALIFICATIONS

1. Program Manager:

Dr. Alfred H. Berner
Group Leader
Farmland Wildlife Populations and Research
Section of Wildlife Populations and Research Unit
Minnesota Department of Natural Resources

Adjunct Professor
Department of Fisheries and Wildlife
University of Minnesota

Ph.D. Wildlife Ecology, Michigan State University, 1969
M.S. Wildlife Ecology, Michigan State University, 1965

Dr. Berner has conducted research and written on a wide variety of subjects but is best known for his work on the impacts of federal farm programs on land use and pheasant populations. He also has experience in refereeing journal articles particularly in the areas of pheasant ecology and management, and impacts of land use on pheasant populations. Dr. Berner's primary role will be to act as Program Manager, and provide advisory and logistic support to the upland portion of this program.

2. Major Cooperators:

A. Dr. David E. Andersen (Uplands)
Assistant Unit Leader - Wildlife
Minnesota Cooperative Fish and Wildlife Research Unit
U.S. Fish and Wildlife Service

Assistant Professor
Department of Fisheries and Wildlife
University of Minnesota

Ph.D. Wildlife Ecology/Zoology, University of Wisconsin, 1988
M.S. Wildlife Ecology, University of Wisconsin, 1984

Dr. Andersen has conducted research on the impacts of human activity on wild birds and other wildlife. His experience includes writing, refereeing, and editing publications in avian ecology and management and working with government agencies concerning mitigation of negative human impacts. Dr. Andersen's primary role will be to conduct the upland portion of the project jointly with Dr. Berner and advising the graduate student who will conduct the upland portion of the project as a Master's project.

B. Dr. Mary G. Henry (Wetlands)
Unit Leader
Minnesota Cooperative Fish and Wildlife Research Unit
U.S. Fish and Wildlife Service

Associate Professor
Department of Fisheries and Wildlife
University of Minnesota

Ph.D. Animal Ecology, Iowa State University, 1984
M.S. Environmental Health, Purdue University, 1978

Dr. Henry has worked extensively in the environmental contaminants field for the past fifteen years. Her experience encompasses bioassessment of contaminant effects on aquatic systems affected by acid precipitation, agricultural insecticides, PCBs, and polycyclic aromatic hydrocarbons. Dr. Henry has conducted this work in association with state and federal agencies and university staff. Her role will be to coordinate and advise graduate students investigating the wetland impacts portion of this project. Dr. Tome will assist in advising graduate students and participate on their committees.

C. Dr. Michael C. Zicus
Wetland Wildlife Populations and Research Group
MN Department of Natural Resources

Ph.D. Wildlife Management, University of Minnesota, 1976
M.S. Wildlife Management, University of Minnesota, 1974

Dr. Zicus has been a waterfowl biologist with the DNR since 1978. He has conducted research and published findings on Canada geese and cavity nesting waterfowl. Since 1985, he also has been a member of the Metropolitan Mosquito Control District's Scientific Peer Review Panel which has advised the district on research regarding the effects of mosquito control on non-target organisms. Dr. Zicus will help coordinate necessary field aspects of the project with DNR wildlife managers.

D. Dr. Michael W. Tome
Wildlife Biologist
North American Waterfowl Management Plan
U.S. Fish and Wildlife Service
340 Arlington Square
Washington, DC 20240

Ph.D. Natural Resource Management, The University of Michigan, 1986
M.S. Wildlife Management, University of Maine, 1982

Dr. Tome has conducted research on waterfowl foraging ecology and energetics, and the impacts of agricultural pesticides on waterfowl and wetlands. Dr. Tome has worked extensively with resource agencies and agriculture extension personnel in identifying agricultural practices that will meet the needs of the farmer while maintaining the quality of wetland habitats. He has written, edited and refereed publications concerning waterfowl ecology and the impacts of contaminants on wildlife. Dr. Tome's primary role will be to assist in coordinating and advising graduate students on the wetland portion of the project with Dr. Henry and serve on the committee of the graduate students who will conduct this portion of the project for Master's degrees.

VI. LITERATURE CITED

Chura, N. J. 1961 Food availability and preferences of juvenile mallards. Trans. N. Am. Wildl. Nat. Res. Conf. 26:121-133.

Coats, J. R., D. M. Symonik, S. P. Bradbury, S. D. Dyer, L. K. Timson, and G. J. Atchison. 1989. Toxicology of synthetic pyrethroids in aquatic organisms: an overview. Environ. Toxicol. and Chem. 8:671-679.

Cooper, J. A., C. M. Potter, and C. H. Welling. 1989. Mallard duckling growth and behavior in methoprene treated wetlands. Unpublished report by the Department of Fisheries and Wildlife, University of MN, St. Paul MN 55018 to the Legislative Commission on Minnesota Resources, State Office Building, St. Paul, MN 55155.

Erpelding, R., R. O. Kimmel, and D. J. Lockman. 1987. Foods and feeding behavior of young gray partridge in MN. Pages 17-30 *in* R. O. Kimmel, J. W. Schultz, and G. J. Mitchell, eds. Perdix IV: Gray partridge workshop. Minn. Dep. Nat. Resour., Madelia.

Grue, C. E., M. W. Tome, T. A. Messmer, D. B. Henry, G. A. Swanson, and L. R. DeWeese. 1989. Agricultural chemicals and prairie pothole wetlands: meeting the needs of the resource and the farmer--U.S. perspective. Trans. 54th N. A. Wildl. and Nat. Res. Conf. 43-58.

Hunter, M. L., J. W. Witham, and H. Dow. 1984. Effects of a carbaryl induced depression in invertebrate abundance on the growth and behavior of American black duck and mallard ducklings. Can. J. Zool. 62:452-456.

Hunter, M. L., J. J. Jones, K. E. Gibbs, and J. R. Moring. 1986. Duckling responses to lake acidification: do black ducks and fish compete? Oikos 47:26-32.

Joselyn, G. B., and G. I. Tate. 1972. Practical aspects of managing roadside cover for nesting pheasants. J. Wildl. Manage. 36:1-11.

Kimmel, R. O., and W. M. Healy. 1987. Imprinting - a technique for wildlife research. Pages 32-57 *in* R. O. Kimmel, J. W. Schultz, and G. J. Mitchell, eds. Perdix IV: Gray partridge workshop. Minn. Dep. Nat. Resour., Madelia.

Lees, P. R., and M. Street. 1974. The feeding ecology of young Mallard (*Anas platyrhynchos*) and tufted duck (*Aythya fuligula*) in wet gravel quarries. Eleventh Internat. Congress of Game Biologists.

Lokemoen, J. T., H. A. Doty, D. E. Sharp, and J. E. Neaville. 1982. Electric fences to reduce mammalian predation on waterfowl nests. Wildl. Soc. Bull. 10:318-323.

Loughrey, A. G., and R. H. Stinson. 1955. Feeding habits of juvenile ring-necked pheasants on Pelee Island, Ontario. Can. Field-Nat. 69:59-65.

Nelson, D. R., R. O. Kimmel, and M. J. Fryendall. 1990. Ring-necked pheasant and gray partridge brood habitat in roadsides and managed grasslands. Pages 103-112 *in* K. E. Church, R. E. Warner, and S. J. Brady, eds. Perdix V: Gray partridge and ring-necked pheasant workshop. Kans. Dep. Wildl. and Parks, Emporia.

Perret, N. G. 1962. The spring and summer foods of the common mallard (*Anas platyrhynchos* L.) in south central Manitoba. M.S. Thesis Univ. of British Columbia, Vancouver.

Southwood, T. R. E., and D. J. Cross. 1969. Ecology of the partridge. III. Breeding success and the abundance of insects in natural habitats. J. Anim. Ecol. 38:497-509.

U.S. Environmental Protection Agency. 1989. Effects, persistence, and distribution of Asana® XL in littoral enclosures. EPA-Duluth, Univ. Wis. Superior. 276pp.

Varland, K. 1985. Why roadsides for wildlife? Minn. Volunteer 2:3-8.

1991 RESEARCH PROJECT ABSTRACT
FOR THE PERIOD ENDING JUNE 30, 1993

This project was supported by the MN Environment and Natural Resources Trust Fund

TITLE:	Insecticide Impact on Wetland and Upland Wildlife
PROGRAM MANAGER:	Alfred H. Berner
ORGANIZATION:	MN Department of Natural Resources
LEGAL CITATION:	M.L. 91 Ch. 254 Art. I Sec.14 Subd: 9(a)
APPROP. AMOUNT:	$650,000

STATEMENT OF OBJECTIVES

The goal of this research was to determine the magnitude of direct and indirect impacts on growth, behavior, and survival of young mallards, pheasants, and gray partridge caused by insecticides used to control agricultural pests (e.g., grasshoppers). Our primary objectives were to determine: 1) the direct impacts of insecticide application on survival of young waterfowl and game birds; 2) the direct effects of insecticides on invertebrates important in the diets of these birds; 3) the indirect effects on growth, survival, and behavior of imprinted mallard and pheasant broods resulting from direct impacts on invertebrate populations.

RESULTS

Wetland Research: In 1991, 1 mallard brood was allowed to forage on 4 wetlands located on U. S. Fish and Wildlife Service (USFWS) Waterfowl Production Areas (WPA's) in western MN. Wetlands were paired on the basis of size (large or small), and 1 wetland in each pair was treated aerially with Asana® XL at the maximum label rate for grasshopper control on non-crop lands. Invertebrate populations were depressed in treated wetlands for up to 15 days after insecticide application and amphipod abundance was reduced to 0. Ducklings were observed foraging on dead invertebrates, and initially gained mass faster on treated than on untreated wetlands. However, this trend was reversed twice over the course of the experiment. Mass gain of ducklings foraging on treated wetlands subsequently was reduced to levels below those observed for ducklings on untreated wetlands. But, by the end of the 15-day experiment, mass gain was similar for both groups of birds. In 1992, 12 imprinted mallard broods were allowed to forage exclusively on either treated or untreated wetlands located on USFWS WPA's in western MN. We observed no significant effect of application of Asana® XL on duckling mass 15 days after treatment, although mean survival for broods reared on treated sites was significantly lower than for broods reared on untreated sites, suggesting that subtle effects of insecticide application on food availability may result in decreased survival and recruitment of ducklings.

Upland Research: In 1991, direct effects on upland game birds were investigated by exposing 2-week old, 6-week old, and ≥ 14-week old ring-necked pheasants and gray partridge to a single field application of Asana® XL, Malathion, or Furadan. Pheasants and partridge exposed to direct application of these insecticides exhibited no obvious signs of acute toxicity to insecticides, and brain cholinesterase (ChE) activity was not depressed in these birds. In July 1992, 2 broods of imprinted ring-necked pheasants were allowed to forage exclusively on upland areas either treated or untreated with Asana® XL located on USFWS WPA's, also in western MN. In all experiments, invertebrate abundance and biomass were reduced following application of insecticides. There was no effect of application of Asana® XL on daily mass change of pheasant chicks, even though both invertebrate number and biomass were reduced on treated plots following insecticide application. Feeding rates of pheasant chicks were not good indicators of growth rates and, similar to ducklings, chicks were observed foraging on dead invertebrates following insecticide application.

PROJECT RESULTS USE AND DISSEMINATION

The results of this research project can be used to guide the application of insecticides as part of routine agricultural practices, and to more fully understand the potential direct and indirect effects of insecticides on non-target organisms. Preliminary results of this project have been presented at the annual meeting of the Society of Environmental Chemistry and Toxicology (SETAC) (November 1992), the Midwest regional chapter of SETAC (October 1991 and March 1993), Northern Prairie Research Center (April 1992), Region 3 of the USFWS (December 1991), the American Society of Limnology and Oceanography (February 1992), North Dakota State University (March 1992), MN Aerial Applicator's Society (March 1992), MN Department of Natural Resources (March 1992 and March 1993), Indiana State University (October 1992), Oklahoma State University (December 1992), Morris and Litchfield Wetland Management Districts of the USFWS (October 1991), and the 54th Midwest Fish and Wildlife Conference (December 1992). Publication in the scientific literature and completion of 2 M.S. theses are anticipated from this project in addition to the final report to the MN State Legislature (see legal citation above).

July 1, 1993

LCMR Final Status Report - Detailed for Peer Review - Research

LCMR EXPANDED WORK PROGRAM

I. Insecticide Impact on Wetland and Upland Wildlife

Program Manager: Alfred H. Berner
Farmland Wildlife Populations and Research
Department of Natural Resources
Rte 1, Box 181
Madelia, MN 56062
507-642-8478

A. M.L. 91 Ch. 254 Art. I Sec.14 Subd: 9(a) Appropriation: $650,000
Balance: $0

Insecticide Impact on Wetland and Upland Wildlife: This appropriation is from the Minnesota (MN) environment and natural resources trust fund to the Commissioner of Natural Resources to research the effect of insecticides on wetland and upland wildlife and habitats.

II. NARRATIVE: State-mandated control of grasshopper outbreaks as well as routine agricultural chemical applications have resulted in spraying of broad-spectrum insecticides on non-cropped wetlands and uplands acres [e.g., roadsides, Conservation Reserve Program (CRP) and Reinvest in MN (RIM)]. Habitat components provided by these acres are critical for the survival of many waterfowl, songbirds, and upland game bird species. Recent studies conducted by Patuxent Wildlife Research Center (PWRC) biologists in North Dakota (ND) documented direct mortality from aerially applied ethyl parathion to waterfowl ducklings and to the aquatic invertebrates that comprise an important component of female waterfowl and duckling's diets. Information is needed to determine how the growth and survival of ducklings that do not die from the insecticide application is affected when their prey base is depleted by insecticide drift or over-spray. By increasing our understanding of the effects of agricultural insecticides on the quality of prairie wetlands, information will be generated to assist wetland managers, farmers, agriculture extension personnel, and state and federal agencies in providing quality prairie wetland habitat while continuing to meet the needs of agriculture. Some insecticides have also been shown to cause direct mortality on upland birds while others have affected populations by reducing or eliminating insects and other invertebrates that comprise the primary food source of young birds. These two complimentary and coordinated study components are designed to determine the extent of direct and indirect insecticide impacts on upland and wetland birds and their invertebrate food base.

A. FINAL ABSTRACT: From 1991 through 1992, we conducted a series of field experiments designed to determine the direct and indirect effects of application of agricultural insecticides on waterfowl and upland game birds.

Wetland Research: In 1991, 1 mallard brood was allowed to forage on 4 wetlands located on U. S. Fish and Wildlife Service (USFWS) Waterfowl Production Areas (WPA's) in western MN. Wetlands were paired on the basis of size (large or small), and 1 wetland in each pair was treated aerially with Asana® XL at the maximum label rate for grasshopper control on non-crop lands. The mallard brood was allowed to forage on 2 pairs of wetlands (1 large pair and 1 small pair) over a 2-day period. Invertebrate populations were depressed in treated wetlands for up to 15 days after insecticide application and amphipod abundance was reduced to 0. Ducklings were observed foraging on dead invertebrates, and initially gained mass faster on treated than on untreated wetlands. However, this trend was reversed over the course of the experiment until rate of mass gain was similar on treated and untreated wetlands.

In 1992, 12 imprinted mallard broods were allowed to forage exclusively on either treated or untreated wetlands located on USFWS WPA's in western MN. We observed no significant effect of application of Asana® XL on duckling mass 15 days after treatment, although mean survival for broods reared on treated sites ($\bar{x}$ = 40.6%, n = 6) was significantly lower than for broods reared on untreated sites ($\bar{x}$ = 65.6%, n = 5) (t = 3.83, 8 df, P = 0.005). Morphological development did not differ between birds allowed to forage on treated versus untreated wetlands.

Upland Research: In 1991, direct effects on upland game birds were investigated by exposing 2-week old, 6-week old, and ≥ 14-week old ring-necked pheasants *(Phasianus colchicus)* and gray partridge *(Perdix perdix)* to a single field application of Asana® XL, Malathion, or Furadan. In both years of the study, indirect effects of insecticides were measured by monitoring direct effects of insecticide application on invertebrate populations important in the diets of upland game bird chicks. In addition to monitoring invertebrate populations, we used imprinted broods ring-necked pheasants (1992) to assess potential indirect effects of Asana® XL application on invertebrate-dependent juvenile birds.

In all experiments, invertebrate abundance and biomass were reduced following application of insecticides. In 1991, pheasant and partridge exposed to direct application of Asana® XL, Malathion, or Furadan did not exhibit any obvious signs of acute toxicity to insecticides, and brain cholinesterase (ChE) activity was not depressed in these birds.

In conjunction with the wetland portion of the study in 1992, 2 broods of imprinted ring-necked pheasants were allowed to forage exclusively on either treated or untreated upland areas located on USFWS WPA's, also in western MN. There was no effect of application of Asana® XL on daily mass change of pheasant chicks, even though both invertebrate number and biomass were reduced on treated plots following insecticide application. Feeding rates of pheasant chicks were not good indicators of growth rates and chicks were observed foraging on dead invertebrates following insecticide application.

Summary: We observed no direct acute effects of field application of Asana® XL on mallard ducklings nor of Asana® XL, Malathion, or Furadan on ring-necked pheasants or gray partridge. Invertebrate populations were significantly reduced following insecticide application in all field experiments, suggesting the potential for indirect effects on ducklings and game bird chicks. Both mallard ducklings and pheasant chicks foraged on invertebrates killed by insecticides, although daily mass changes were not different between birds that foraged on treated versus untreated areas in 1992. In the case of mallards, brood survival was reduced as a result of aerial application of Asana® XL and mediated by direct impacts on invertebrate populations, suggesting that subtle effects of insecticide application on food availability may result in decreased survival and recruitment.

III. OBJECTIVES:

A. Determine the extent of direct and indirect mortality on young mallards, pheasants, and gray partridge caused by chemicals used to control insects (e.g., grasshoppers).

A.1.1. Narrative (wetlands): Determine the extent of direct and indirect mortality in imprinted mallards and their associated wetland invertebrate food base from ~~chemicals~~ Asana ® XL used at recommended rates to control crop insect pests.

This wetland portion of the project will focus on mallard ducklings and aquatic invertebrates.

A.1.2. Narrative (uplands): Determine the extent of direct and indirect mortality on pen-enclosed ring-necked pheasants and gray partridge and their associated invertebrate food base from chemicals used to control insect crop pests.

This upland portion of the project will focus on gray partridge, pheasants, and terrestrial invertebrates.

A.2.1. Procedures (wetlands):

A.2.1.1. Protocol Development: Utilizing available information generated by the ~~U.S. Fish and Wildlife Service~~ USFWS, ~~Patuxent Wildlife Research Center~~ PWRC, and information available in the literature, details of the research protocol ~~was finalized~~ were completed. This research ~~is being~~ was conducted under faculty supervision by 1 graduate degree student in Wildlife Conservation at the University of Minnesota (U of MN) and 1 staff scientist; these people ~~was~~ were responsible for coordinating the majority of the field work. ~~Part of the duties of these people was to refine the project design and protocol outlined below.~~

A.2.1.2. Study Site: The field portion of this study began April 1991 and was concluded August 1992. In the first field season, we selected 4 wetlands on federal ~~Waterfowl Production Areas~~ WPA's of similar type, size, biotic character, and surrounding land-use pattern. Wetlands were paired on the basis of proximity, size, and type. ~~and 1~~ One wetland in each pair served as a reference while the second served as a treated wetland. In 1992, we selected 12 study wetlands following the approach used in the previous year. The study was designed to monitor the quality of the wetland ecosystem as waterfowl brood-rearing habitat. Not only did we measure different aspects of the wetland ~~ecosystem~~ to determine the impact insecticides may have had on the ecology of this ecosystem, but, we also studied the growth and behavior of broods released onto the wetlands ~~so that we might~~ to demonstrate ~~obtain further information on~~ the potential sub-lethal impacts insecticides might have on waterfowl.

A.2.1.3. Insecticides: Asana® XL, a synthetic pyrethroid insecticide that is ~~very~~ highly toxic to invertebrates (Coats et al. 1989, U.S. Environmental Protection Agency 1989) but is essentially non-toxic to birds and mammals, was applied by a licensed aerial applicator. The deposit of insecticide in the wetland was measured ~~with~~ using 3 circular filter papers (spray deposit cards) ~~similar to those used in previous North Dakota and MN pesticide studies,~~ on stakes located at each of the invertebrate sampling stations. This method is identical to the methods used previously in ND and MN pesticide studies.

A.2.1.4. Experimental Design:

Impact of insecticide spraying on selected aquatic invertebrate populations: Three transects were established in each wetland. Each transect began in the center of the wetland and radiated at an angle of 120° ~~angles towards the shore~~ from other transects.

~~A~~ Two permanent invertebrate sampling stations were established along each transect at the open water/deep marsh zone interface, and in the shallow marsh zone.

Invertebrates important in the diet of young ducklings were sampled in 1991 and 1992. In 1992, we sampled aerial insects that occurred in the area accessible to foraging ducklings using emergence traps. An emergence trap consists of a floating frame from which a mesh tent is suspended. Aquatic insects emerging into this trap are funneled toward the top of the tent and trapped in a collection jar filled with a preservative. We ~~then~~ identified each invertebrate (at least to Family) and weighed taxonomic groups to obtain an estimate of the types of invertebrates and biomass available to ducklings foraging ~~on~~ at the water surface. ~~Three~~ Six emergence traps were placed on each wetland. The traps were ~~placed~~ set in the wetlands ~~at~~ 1 day ~~pre-spray~~ prior to, and collected 1 day ~~post-~~ subsequent to spray, and then set and collected at weekly intervals until 28 days post-spray. ~~Aquatic~~ Invertebrate populations occurring in the water column were sampled during the season with activity traps (3 6 traps/wetland) and 5 cm ~~dia.~~ diameter core samplers (3 cores/wetland). Aquatic invertebrates were sampled 1 day pre-spray, 1 day post-spray, at 3-day intervals for the first 15 days post-spray, and at weekly intervals ~~for~~ from 15 to 28 days post-spray. We compared numbers, species, and biomass of invertebrates collected during the same time intervals ~~between~~ in treated and reference wetlands and compared differences using 1 way analysis of variance (ANOVA) and 2 sample t-tests.

In addition, the effect of Asana® XL on invertebrates was also evaluated ~~with~~ using in-situ field bioassays during 1991 and 1992. This involved placing cages containing known numbers of clean, same age, laboratory cultured amphipods and chironomids larvae in ~~the~~ each study wetlands ~~4~~ 1 hours before application of the insecticide. Cages ~~are~~ were then recovered within 4 hours after spray and the survival of the organisms in them ~~is~~ was ~~assessed~~ determined.

Growth and behavior of ~~wild strain~~ game farm mallards released onto study wetlands: This portion of the study was conducted in 1992, while a pilot study was conducted in 1991. The effect of Asana® XL on waterfowl was evaluated using mallard ducklings imprinted on human observers. Imprinted ducklings have been used previously to assess impacts of low pH (Hunter et al. 1986) and ~~other~~ insecticides (Hunter et al. 1984, Cooper et al. 1989) on wetland wildlife. Ducklings were ~~introduced~~ allowed to forage on study wetlands ~~approximately~~ 5 from 1 to 2 days before the insecticide was applied to treated wetlands. Each duckling was individually marked with colored flagging tape and numbered web tags, weighed daily, and ~~measured~~ measures of wing, culmen, and tarsus were recorded at ~~5-day~~ 3-day intervals.

Large predator-proof fences (Lokemoen et al. 1982) were not used to enclose wetlands as these are designed to enhance natural waterfowl nest success on upland sites. These large fences have been found to be ineffective in predator-proofing wetlands ~~,as.~~ Any mink *(Mustela vison)* that are able to pass the barricade can establish a den in the wetland area and prey upon the eggs of hens that nest within the enclosure (J. T. Lokemoen USFWS, Northern Prairie Research Center, pers. comm.). Our study ~~is~~ was designed to determine the impact of pesticide-induced perturbations on aquatic invertebrates and on the subsequent growth and survival of ducklings. For our study purposes, ducklings imprinted on human observers were allowed to forage on the wetlands under the surveillance of ~~the~~ human observers. This ~~allows for~~ approach facilitated daily weighing, ~~and~~ while the presence of the observer on the wetland (acting as a hen) deterred predation. Reliance on natural reproduction is too risky and does not allow for daily weight gain/loss determinations.

Behavior of broods on treated and reference wetlands was monitored using scan sampling techniques. The behavior of individual ducklings (i.e., rest/sleep, ~~comfort movements~~, locomotion, searching for food, foraging above ~~water, on water surface~~, or below water surface) was ~~will be~~ recorded ~~onto~~ with the use of laptop computers. In addition, brood location (i.e., ~~shallow marsh, deep marsh~~ emergent vegetation, open water, or upland) on ~~the~~ each study wetland was ~~will be~~ noted. We ~~are~~ were primarily interested in gross differences in the ability of ducklings to forage efficiently, ~~and~~ leading to potential impacts on growth rates differences between broods reared on treated and reference wetlands ~~during the different time periods~~ due to changes in the invertebrate forage base as a result of insecticide introduction into the wetland. ~~This~~ These differences ~~will be~~ were ~~detected~~ tested using ~~ANOVA's and, if necessary, the appropriate multiple comparison~~ 2 sample t-tests. ~~Their~~ Differences in weight and morphometry ~~will be~~ were compared between birds reared on treated and reference ~~control~~ wetlands with 2 sample t-tests.

Laboratory Toxicity Tests: In order to completely assess the toxicity of Asana® XL to aquatic invertebrates, controlled laboratory studies ~~will be~~ were conducted using ~~representative, indigenous invertebrates~~ 2 laboratory cultured species, both of which are commonly found in wetlands and important food items for ducklings. They were exposed to the same formulation and concentration of Asana® XL as applied under field circumstances. By controlling temperature, light source, food base and dissolved oxygen, the biological impact of the chemical can be ascertained when all other

~~variables~~ conditions are ~~maximized~~ optimized and held constant. Mortality ~~and molting success will be~~ was evaluated in flow-through toxicity tests. ~~Additional~~ Screening of field collected sediment pore water (a reservoir for many aquatic contaminants) ~~will be~~ was also assessed using MICROTOX®, a bioluminescing bacteria that reacts to toxic concentrations of contaminants in water. This laboratory work ~~will be dovetailed into~~ confirmed mortality patterns seen in field results. ~~and the two sets of data will be compared for toxicity implications.~~

A.2.2. Procedures (uplands):

A.2.2.1. Protocol Development: Initially, a comprehensive literature review was conducted and details of the research protocol were finalized based on published literature and study site restrictions and logistics. This research ~~is being~~ was conducted under faculty supervision by 1 Master of Science degree students in Wildlife Conservation at the ~~University of Minnesota~~ U of MN, and the student ~~will be~~ was responsible for coordinating the majority of the field work.

A.2.2.2. Study Site: Field research in 1991 was conducted at the ~~Minnesota~~ MN Department of Natural Resource's (DNR) Farmland Wildlife Populations and Research (FWPR) facility near Madelia, MN. A 20-acre upland grass-legume field was manipulated to provide habitat plots that were used in a field experiment. ~~Plots were discretely bounded by mowing strips between adjacent plots (Fig. 1).~~ Habitat plots in 1991 were similar in vegetative cover to roadsides, CRP, and RIM habitat and were approximately 16 m wide and 25 m long. Ten blocks of 5 plots each were created by mowing buffer zones of 8 m between adjacent plots and between plots and nearby habitat (Fig. 1). Upland habitat dominated by cool-season grasses and legumes is important brood habitat for both pheasants and gray partridge in south-central MN (Nelson et al. 1990) and is similar to roadside habitats that comprise a significant proportion of non-agricultural habitats in the region (Joselyn and Tate 1972, Varland 1985).

Field research in 1992 was conducted on upland areas ~~1 to 5 acres~~ up to 2 ha in size on ~~U. S. Fish and Wildlife Service Waterfowl Production Areas~~ USFWS WPA's associated with wetlands ~~near Willmar~~ in western, MN. These areas had been seeded previously with warm-season native grasses but cool season grasses and legumes were also present

A.2.2.3. Insecticides: In 1991, we selected 3 broad-spectrum insecticides that vary in toxicity to birds (as measured by the lethal dose to 50% of test organisms (LD_{50}'s) reported in the literature) and that are commonly applied to agricultural land in MN. The 3 categories of insecticides, based on toxicity were: $LD_{50} < 20$ mg active ingredient (ai)/ kg body weight (~~e.g.,~~ Furadan), LD_{50} between 100 and 300 mg ai/ kg body weight (~~e.g.,~~ Malathion), and $LD_{50} > 2000$ mg ai/ kg body weight (~~e.g.,~~ Asana® XL). All pesticides selected were water soluble to ~~avoid~~ minimize possible confounding effects of carriers, and are ~~were~~ registered for use on agricultural land and crops commonly grown in ~~Minnesota~~ MN.

In 1991, insecticides were applied from the ground with a hydraulic boom-sprayer pulled behind a vehicle. A plot width of 16 m allowed each plot to be sprayed with 2 passes of the sprayer~~, one on each side of the plot, thus avoiding physical disturbance to plots as a result of application techniques~~. Insecticides were applied at label rates and application rates were determined by mixing known amounts of insecticides with water in the application tank and by ~~analysis~~ analyzing ~~of~~ spray cards placed in habitat plots prior to spraying. Drift from one plot to another was minimized by applying the insecticides when environmental conditions were optimal for spraying (e.g., no wind).

In 1992, the broad-spectrum insecticide Asana® XL was sprayed on the study areas in conjunction with the wetland portion of the study. This insecticide is registered for use on cropland ~~in MN~~ and is commonly used on agricultural areas in MN ~~in this state~~. Asana® XL was aerially applied at a rate recommended for grasshopper control in ~~Minnesota~~ MN.

A.2.2.4. Experimental Design: In 1991, insecticides were applied twice during the course of the experiment to approximate ~~normal~~ standard application practices in the region, and to evaluate both the direct and indirect effects of the insecticides. The 10 habitat blocks (Fig. 1) were divided into 2 groups of 5 blocks. One group of 5 blocks was sprayed in mid-June and both groups were sprayed in mid-July. Within each block, application of each insecticide, application of a water control, plus an untreated control plot, were assigned randomly, for a total of 5 plots per block, and 5 replications ~~of~~ within each block. An additional plot was added to each replicate block to reduce the probability that plots randomly assigned to the same treatment would be adjacent to one another (Fig. 1).

In mid-June 1991, 1 group of replicate blocks was sprayed and insect populations were monitored by sweep netting prior and subsequent to spraying ~~and post-spraying~~ at ~~regular~~ 5-8 day intervals. In mid-July, both groups of blocks were sprayed and 5 pheasants and 5 gray partridge of 3 age classes (2 weeks, 6 weeks, and ≥14 weeks) were placed in each plot in the groups of blocks that were not sprayed in June.

Pheasants and gray partridge were directly subjected to spraying. Insect populations were monitored in this group of blocks prior to and after insecticide application.

Indirect effects on game birds were investigated by monitoring direct impacts on insect populations, which comprise the majority of food for chicks through their first 5-6 weeks of life (Loughrey and Stinson 1955, Southwood and Cross 1969, Erpelding et al. 1987). Sweep net samples were collected at ~~multiple~~ 2 sites within each plot (e.g., Nelson et al. 1990) and insects ~~are being~~ were identified to Order, Family, or Genus, depending upon ease of identification and importance in the diet of pheasants and gray partridge as determined from ~~available~~ published literature. Insect samples ~~are being~~ were oven dried and weighed to determine mass.

Direct effects on game birds were investigated by monitoring behavior and mortality for up to ~~4 weeks~~ 14 days post-spray~~ing~~. Birds were removed from study plots within 1 hour ~~post-spraying~~ after insecticide application and monitored in holding pens. Zero to 5 birds per age class per species per treatment were euthanized at 1, 4, 7, 10, and 14 days after spraying. Brain ~~acetylcholinesterase~~ ChE activity ~~will be~~ was determined from a random subsample of ~~the~~ euthanized birds and all birds that died subsequent to spraying.

In 1992, Asana® XL was applied twice during the course of the experiment ~~in 1992~~. Two upland areas were treated in mid-June and ~~two~~ 2 were treated in mid-July. Two broods consisting of 10 ring-necked pheasant chicks were imprinted ~~to~~ on human observers and were allowed to feed exclusively on either insecticide-treated or untreated study areas. ~~Monitoring the birds continued until 15 days post-spray.~~ Birds were monitored up to 15 days post-spray. Two additional groups of 5 ring-necked pheasant chicks fed alternately on the treated and untreated areas ~~to provide additional information~~.

Indirect impacts of the insecticide on pheasants ~~will be~~ were measured by comparing differential ~~weight gain, if any,~~ mass change for each brood and by monitoring feeding rates of the broods. Sweep net samples were collected 4 times daily in the areas in which chicks were actively feeding to correlate with mass ~~gain~~ change and feeding rates. Insect samples ~~are being divided~~ were identified and placed into taxonomic groups important in the diet of young pheasants and oven dried and weighed to determine biomass.

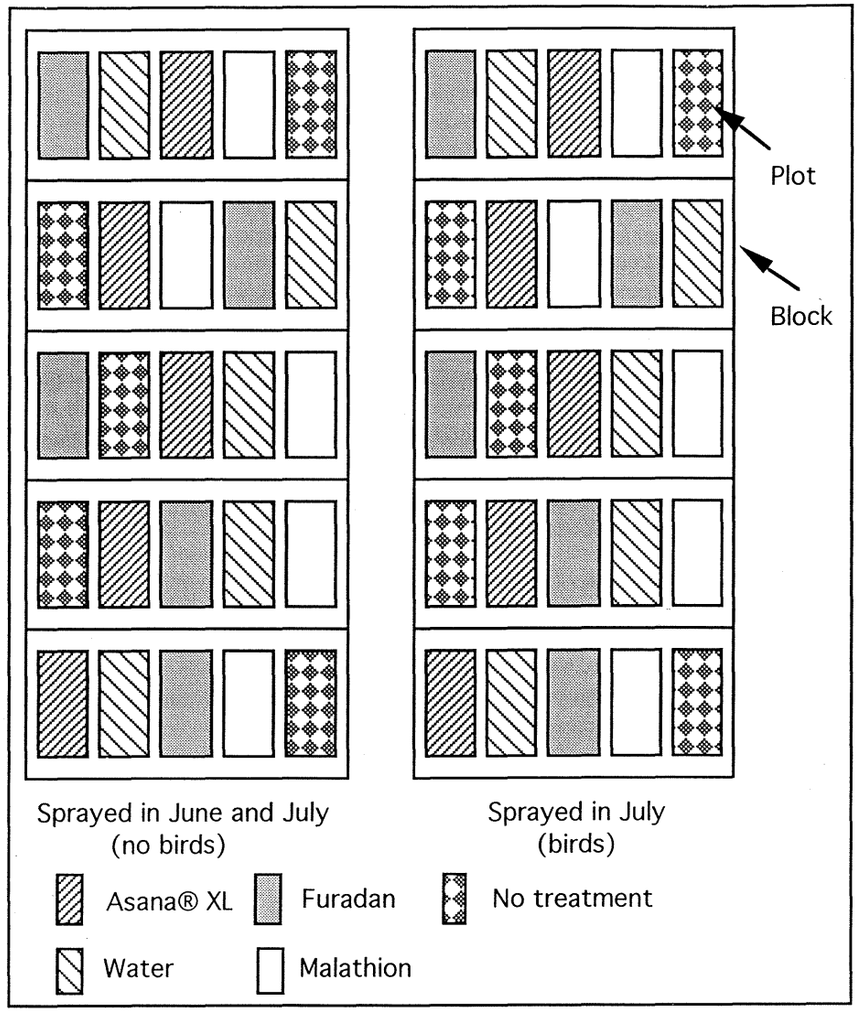


Fig. 1. ~~Revised~~ Study design indicating plots, blocks, replicate blocks, and spraying pattern in 1991.

A.2.2.5. Follow Up Studies: Based on the results of the 1991 experiment, we further evaluated the indirect effects of agricultural insecticides on upland game birds in 1992 as described above. In conjunction with the wetland portion of this project, we compared the growth and feeding rates of imprinted pheasant broods (Kimmel and Healy 1987) in upland habitats associated with wetlands ~~in~~ on both ~~the spray~~ treated and untreated reference areas ~~control group of wetlands selected for study~~. Sweep net samples of insects were collected prior to and post-spray~~ing~~. Sweep net samples were also collected in the immediate vicinity of feeding broods.

A.3.1. Budget (wetlands):

	LCMR Funds
a. Amount Budgeted:	$500,000
b. Balance:	$0

A.3.2. Budget (uplands):

	LCMR Funds
a. Amount Budgeted:	$150,000
b. Balance:	$0

A.4.1. Timeline for Products/Tasks (wetlands):

	July91	Jan92	Jun92	Jan93	Jun93
Design details of experiment	. . . . .				
Select experimental wetlands	. . . . .				
Monitor invertebrates	. . . . .				
Monitor behavior of imprinted ducklings	. . . . .		. . . .		
Analyze year 1 (pre-spray) data make design changes		. . . . .			
Set out spray cards, hire contract spray, collect water samples, monitor pre and post spray change in waterfowl and invertebrates	. . . . .		. . . . .		
Pick and identify invertebrates in samples	. . . . .			. . . . .	
Data analysis, write-up				. . . . .	

A.4. 2. Timeline for Products/Tasks (uplands)

	July91	Jan92	Jun92	Jan93	Jun93
Prepare detailed study plan	. . . . .				
Prepare study plots	. . . . .				
Prepare Madelia experiment	. . . . .				
Conduct Madelia experiment	. . . . .				
Analyze experiment results prepare report for year 1		. . . . .		. . . . .	
Prepare study plots for roadside experiment		. . . . .			
Conduct follow up experiment			. . . . .		
Final report preparation					. . . . .

A.5.1. Status (wetlands):

A.5.1.1. Protocol Development: In 1991, a pilot study was begun in ~~March~~ April with funds from the USFWS ~~U.S. Fish and Wildlife Service~~. The effect of Asana® XL on waterfowl was evaluated using a single brood of mallard ducks imprinted on human observers and maintained on ~~a number of~~ 4 wetlands (Fig. 2). This approach was used in a previous study ~~of~~ investigating the relationship between presence of fish and intake of food by ducklings (Hill et al. 1987).

In 1992, the effect of Asana® XL on waterfowl was evaluated using ~~a number of~~ 12 broods of mallard ducks imprinted on human observers; each brood was maintained on ~~one~~ an individual study wetland (Fig. 3).

A.5.1.2. Study Site: Study sites were located on ~~U.S. Fish and Wildlife Service Waterfowl Production Areas~~ USFWS WPA's in the Minnesota Waterfowl and Wetlands Management Complex (MWWMC). We avoided wetlands connected by channels ~~of flowing water~~ to other basins to minimize potential influences of water movement on effects of ~~pesticide~~ insecticide application ~~to study wetlands~~. We also avoided wetlands known to have fish, which can compete with ducklings for food (Hill et al. 1987). Potential study sites were sampled for fish with activity traps (Murkin et al. 1983) and those with fish were rejected. ~~In one wetland selected for study in 1992, after no fish were captured in activity traps, fish were subsequently seen by project staff.~~

In 1991, research was conducted on ~~four~~ 4 ~~semi-permanently flooded~~ palustrine, persistent-emergent (dominance types: *Typha* spp. and/or *Scirpus* spp.) (Cowardin et al. 1979), or Type 4 wetlands (Shaw and Fredine 1956) in Pope County. Two of

these wetlands were 2 to 2.5 ha in size and classified as small; the other ~~two~~ 2 were 4 to 4.5 ha in size and classified as large. A randomized block experimental design was used; wetlands were assigned to blocks based on size. Wetlands to which Asana® XL was applied are considered 'treated' and those to which no insecticide was applied are referred to as 'reference' wetlands.

In 1992, we selected ~~twelve~~ 12 palustrine, persistent-emergent (dominance types: *Typha* spp. and/or *Scirpus* spp.) (Cowardin et al. 1979), or Type 4 wetlands (Shaw and Fredine 1956) located in Kandiyohi, Stearns, Swift, and Pope Counties, for use in a second randomized block experiment. Each block consisted of ~~four~~ 4 wetlands that were studied simultaneously during ~~three~~ 3 different months~~,~~ : May, June and July. In each block, ~~two~~ 2 wetlands were randomly ~~chosen~~ selected to be treated with Asana® XL; the other ~~two~~ 2 wetlands ~~were randomly chosen to~~ served as ~~untreated,~~ reference wetlands. An exception to this design occurred in the third block when a wetland was randomly selected to be treated but was subsequently made a reference wetland because high water made it contiguous with an adjacent wetland separated from the study wetland by a road. In this wetland, after no fish were captured in activity traps during preliminary sampling used for site selection; fish were subsequently seen by project staff.

A.5.1.3. Insecticide: Asana® XL, was aerially applied on 10 June 1991 at the nominal rate of 34 g ~~active ingredient ha^{-1}~~ ai/ha, the maximum label rate for grasshoppers on non-crop land (Anonymous 1991). ~~The average rate of deposition on spray cards in the treated wetlands was slightly greater than the nominal rate.~~

In 1992, Asana® XL was aerially applied to wetlands in the first block on 20 May ~~1992~~ at the nominal rate of 34 g ~~active ingredient ha^{-1}~~ ai/ha, the maximum label rate for grasshoppers on non-crop land (Anonymous 1991). Applications were made at the same rate on wetlands in the second and third blocks on 18 June and 15 July, respectively.

Fig. 2. The experimental design used in our 1991 field season consisted of 4 semi-permanent wetlands paired based on size (large and small). A single brood of imprinted mallard ducks was allowed to forage freely for 2, 6.5 hour periods daily. When a forage period had been completed the ducks were recovered and weighed. The brood was moved at mid-day to the second wetland in the pair. This process was repeated daily and resulted in equal time spent on both reference and treated sites. Behavioral observations were collected while the birds were on the wetlands. Equal time was spent on all 4 wetlands within any 2-day block. Forage bouts were randomly assigned based on wetland pairs and treatment.

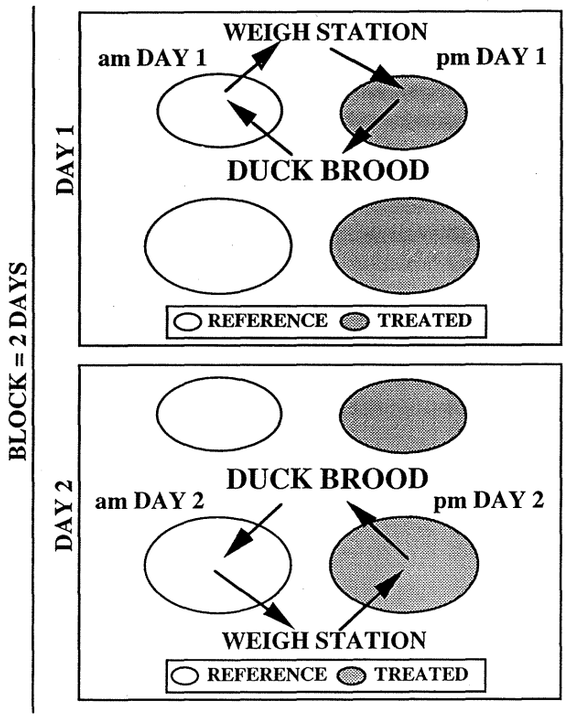
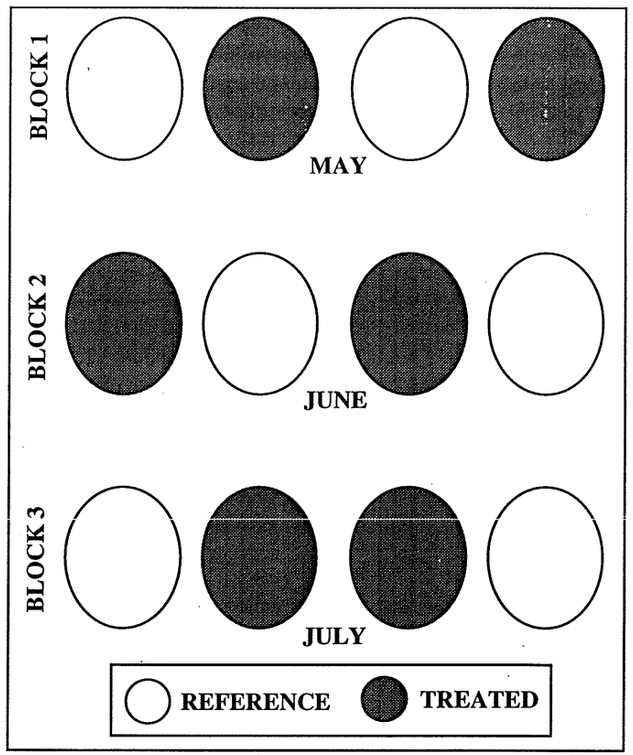


Fig. 3. The experimental design used in 1992 consisted of 12 wetlands selected and grouped into 3 blocks of 4 wetlands each. Treatment was randomly assigned to 2 wetlands in each block. One brood of mallard ducks was randomly assigned to, and reared on, each wetland for 15 days post-treatment.



A.5.1.4. Results and Discussion

Insecticide: The average rate of Asana® XL deposition on spray cards in the treated wetlands was slightly greater than the nominal rate.

Impact of insecticide spraying on selected aquatic invertebrate populations: In the treated wetlands during 1991, the total number of invertebrates captured in activity traps declined immediately following application of Asana® XL. This decline persisted for approximately 11 days, at which point the recovery of invertebrate abundance had begun (Fig. 4). The portion of the invertebrate community sampled in activity traps returned to abundance levels similar to those sampled in reference wetlands within approximately 18 days post-treatment, with the notable exception of amphipods, which were essentially eliminated following treatment. In benthic cores, numbers of chironomid larvae were not reduced by the application of Asana® XL during 1991. Numbers of amphipods in cores were reduced to 0 immediately following treatment. Amphipods were still not present in either activity trap or benthic core samples collected from treated wetland sites 1 year later.

The effect of Asana® XL on cultured invertebrates was also evaluated with in-situ field bioassays. Both amphipods and chironomid larvae in these bioassay cages experienced significant acute mortality due to application of Asana® XL. However, amphipods demonstrated a greater degree of sensitivity to Asana® XL than chironomids (Table 1). These results confirm the treatment response we observed in the naturally occurring invertebrates from these taxonomic groups when treated with Asana® XL.

In 1992, numbers of invertebrates in activity traps in treated wetlands declined immediately after application of Asana® XL and remained at levels lower than those observed in untreated wetlands for the rest of the 15 day post-treatment time period (Fig. 5). Insects captured in emergence traps consisted primarily of chironomids, which occurred in 99% of the samples collected and constituted 91% of the overall mean number of insects in samples. In treated wetlands, the number of emerging insects, including numbers and biomass of chironomids declined immediately after application of Asana® XL and remained at levels lower than those observed in untreated wetlands throughout the 15 day post-treatment time period (Figs. 6, 7, and 8).

Fig. 4. Total number of invertebrates captured in activity traps in western MN during 1991 (reference $n = 2$, treated $n = 2$). Treatment (spray) was an aerial application of Asana® XL to 2 wetland study sites.

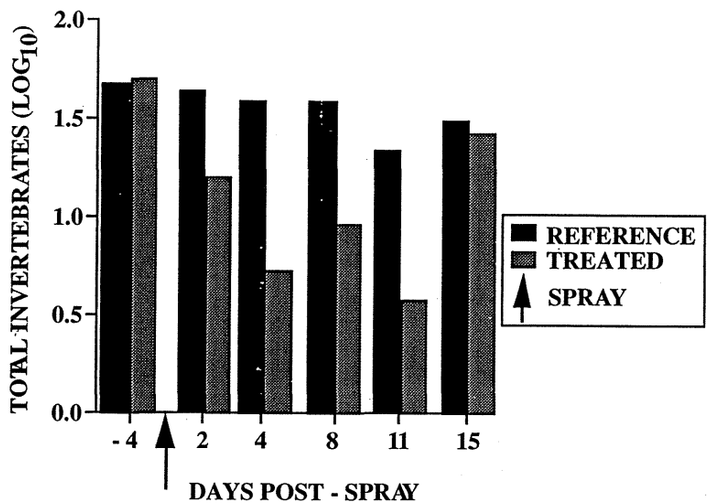


Table 1. In-situ field bioassay results for Asana® XL research conducted in western MN during 1991 and 1992. ANOVA was used to test for treatment effects on survival; sample sizes for 1991 and the 1992 block analyses were reference $n = 2$ and treated $n = 2$. Total sample size for 1992 was $n = 6$ for reference and $n = 6$ for treated wetlands.

		Chironomus tentans larvae				*Hyallela azteca*			
Year	Group	Dead	Alive	Missing	P-value	Dead	Alive	Missing	P-value
1991									
	Reference	8	107	5		4	107	9	
					0.0548				0.0008
	Treated	38	72	10		114	0	6	
1992									
	Block 1								
	Reference	43	174	26		31	153	58	
					0.1140				0.0274
	Treated	159	54	36		186	5	49	
	Block 2								
	Reference	17	344	4		56	257	48	
					0.9362				0.0309
	Treated	37	341	6		324	7	39	
	Block 3								
	Reference	57	252	13		42	197	72	
					0.1016				0.0158
	Treated	139	186	25		279	5	67	
1992	**Totals**								
	Reference	117	770	43		129	607	178	
					0.0415				0.0002
	Treated	335	581	67		789	17	155	

Fig. 5. Total number of invertebrates captured in activity traps from 6 treated and 6 reference wetlands in western MN in 1992. Research was conducted in 3 blocks during a 3-month period. Each block consisted of 2 reference and 2 treated wetland study sites. Treatment (spray) was a single aerial application of Asana® XL to wetland study sites on 20 May, 18 June, or 15 July 1992.

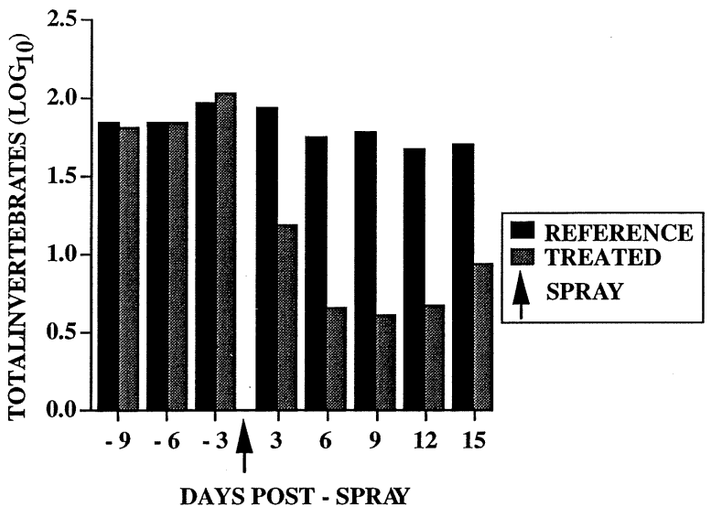


Fig. 6. Total number of insects captured in emergence traps from 6 treated and 6 reference wetlands in western MN in 1992. Research was conducted in 3 blocks during a 3-month period. Each block consisted of 2 reference and 2 treated wetland study sites. Treatment (spray) was a single aerial application of Asana® XL to wetland study sites on 20 May, 18 June, or 15 July 1992.

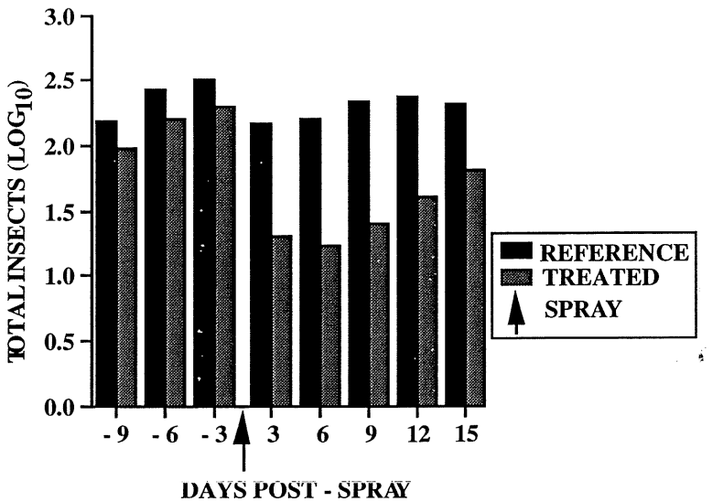


Fig. 7. Total number of chironomids captured in emergence traps from 6 treated and 6 reference wetlands in western MN in 1992. Research was conducted in 3 blocks during a 3-month period. Each block consisted of 2 reference and 2 treated wetland study sites. Treatment (spray) was a single aerial application of Asana® XL to wetland study sites on 20 May, 18 June, or 15 July 1992.

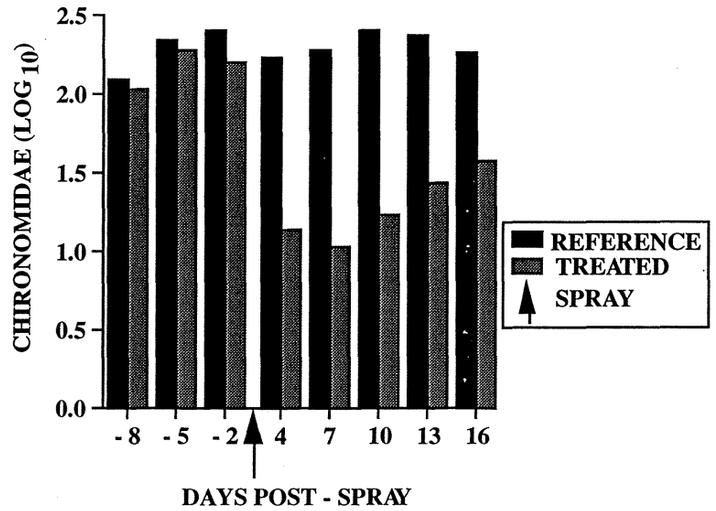
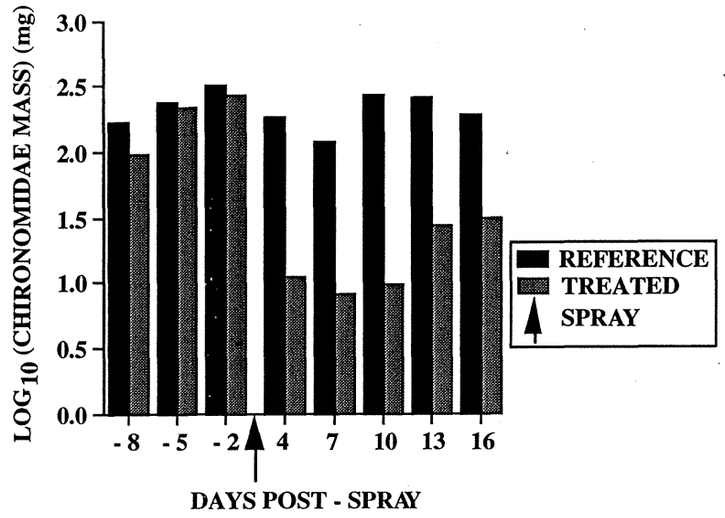
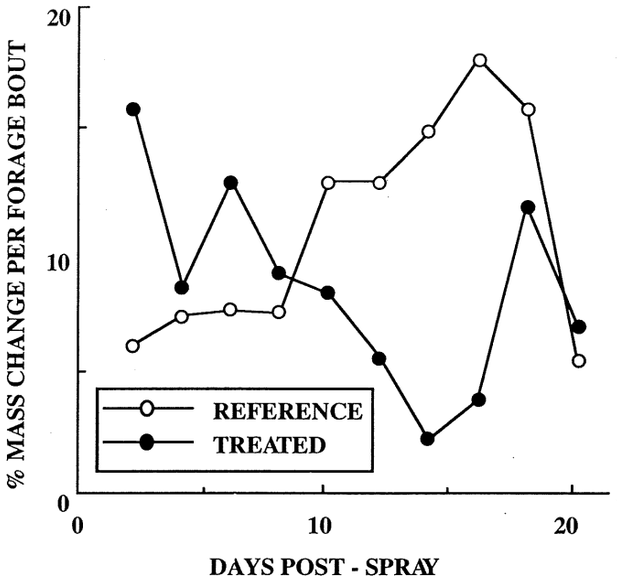


Fig. 8. Wet mass (mg) of chironomids captured in emergence traps from 6 treated and 6 reference wetlands in western MN in 1992. Research was conducted in 3 blocks during a 3-month period. Each block consisted of 2 reference and 2 treated wetland study sites. Treatment (spray) was a single aerial application of Asana® XL to wetland study sites on 20 May, 18 June, or 15 July 1992.



Growth and behavior of mallard ducklings released onto study wetlands -1991: No duckling mortality was observed due to direct toxicity of Asana® XL. Immediately after application of Asana® XL, duckling mass gain per feeding session was higher on treated wetlands than on reference wetlands. Duckling mass gains that occurred on treated and reference wetlands were nearly equal at 8 days post-treatment, however, birds gained more mass when they foraged on reference compared to treated sites during the 9 to 17 post-spray time period. Finally, by 18 to 20 days after spray, mass gains were nearly equal for forage bouts on both reference and treated wetlands (Fig. 9). In the case of a single mallard brood allowed to forage on multiple wetlands, as in this study, change in mass per feeding session is an indication of short-term food intake as opposed to long-term growth. A summary of behavioral observations indicated that the activity budgets of ducklings on the reference wetlands were consistent throughout the duration of the study. In contrast, the feeding and resting patterns of ducklings on the treated wetlands varied. During the 6 days immediately after application of insecticide, ducklings foraged less and rested more on

Fig. 9. Percent mass change per controlled forage bout for a single brood of imprinted mallard ducks. Forage bouts were conducted on 4 semi-permanent wetlands (2 treated 2 reference) located in Pope County MN in 1991 . Treatment (spray) was a single aerial application of Asana® XL on 10 June 1991.



treated wetlands than they did on reference wetlands. This trend was reversed from 8 to 12 days after Asana® XL application.

Growth and behavior of mallard ducklings released onto study wetlands, block 1 May 1992: The daily mass changes of birds reared on both reference and treated wetlands were influenced by ambient temperatures recorded during block 1. Daily mean percent mass change per brood was strongly correlated with mean daily temperature (all r's $\geq$ 0.83, all P's $\leq$ 0.01). During the first 4 days post-treatment we observed 4 ducklings from a single treated wetland that apparently were unable to maintain sufficient mass gain to survive. The activities of this brood during this time period were marked by constant distress calling and increased time spent on food searching behaviors, including searching activities in the upland habitat adjacent to the wetland. This brood experienced 100% mortality at 5 days post-spray (25 May). The mean daily mass for all birds increased for 2 days following insecticide application (20 May), followed by 3 days of consistent mass loss for all birds. Surviving broods were sheltered and provided supplemental food from noon on 25 May to noon on 27 May due to inclement weather and were subsequently allowed to forage exclusively on wetlands for the remaining 9 days of this block. The mean duckling weights for all of the surviving broods were not different (reference $\bar{x}$ = 55.9 and 56.0, treatment $\bar{x}$ = 56.1) following the first full day (27 May) that they returned to foraging exclusively on wetlands. However, the mean daily mass of the surviving treatment brood remained consistently lower than the 2 reference broods for the remainder of this block. Mean brood weights, (at 15 days post-treatment) for block 1 were significantly different for birds reared on treated (n = 7, $\bar{x}$ = 66.6 g) versus reference wetlands (n = 13, $\bar{x}$ = 84.7 g) (t = -2.73, 18 df, P = 0.014). Individual duckling survival at 15 days post-treatment for block 1 was significantly higher for birds reared on reference wetlands (52%) than for birds reared on treated sites (25%) (χ^2 = 4.10, 1 df, P = 0.043).

Block 1 experimental design changes: Research was interrupted from noon on 25 May to noon on 27 May due to temperatures that ranged from 6.1 to 10.5 C below normal (G. Spoden MN DNR, Midwestern Climate Center, pers. comm.). Prior to this time period we observed 4 days of consistent mass loss that we felt were weather related and threatened duckling survival. In an effort to preserve the integrity of the experimental design of block 1, we sheltered and provided supplemental food to all surviving birds until the weather improved sufficiently to allow their return to wetlands.

Growth and behavior of mallard ducklings released onto study wetlands, block 2 June 1992: Mean daily masses for 2 broods reared on treated wetlands and 1 brood reared on a reference wetland did not differ throughout the course of block 2 until the final sampling day. Mean brood masses for block 2, at 15 days post-treatment, were significantly different for birds reared on treated (n = 13, $\bar{x}$ = 220.8 g) versus reference wetlands (n = 7, $\bar{x}$ = 182.9 g) [t = 2.35, 18 df, P = 0.031; note that an F-test for equal variances ($F_{6,12}$ = 3.50, P = 0.031) indicated that the variances were not equal - - this inequality may result in the appearance of a more powerful t-test than if the variances were equal]. Individual duckling survival at 15 days post-treatment for block 2 was significantly higher for birds reared on the reference wetland (100%) than the 2 treated sites (56%) (χ^2 = 6.24, 1 df, P = 0.012). Nine birds were lost and never recovered. These losses are likely attributable to mink depredation. Observations of gut contents obtained from birds during block 2 revealed that all birds in this block were consuming large quantities of vegetative matter, primarily seeds at a much earlier age than has previously been reported in the literature (Lees and Street 1977, Perret 1962, Chura 1961).

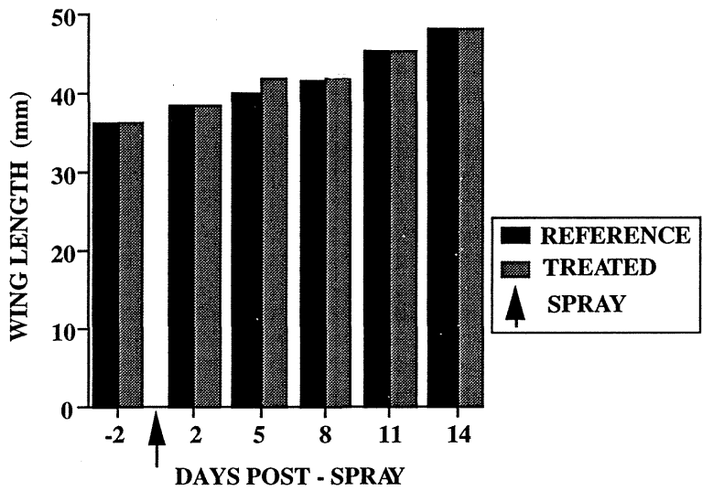
Block 2 experimental design changes: Cold weather curtailed the morning forage bout for all of the broods on 20 June, until a minimum 10 C air temperature was reached. A brood of birds originally included in block 2 and assigned to a reference site failed to maintain sufficient body mass to insure survival. This condition may have led to the unusually high mortality experienced by this brood through the first 4 days of this block. As a result, all broods in block 2 received supplemental food for 3 evenings during the time when the birds were not on the wetlands. The brood with lower body mass was provided an additional 2 days of supplemental food when they were off the wetland, at which point we removed these birds from this reference site and relocated them to a reference site used in 1991 for the remaining 11 days in block 2. This brood appeared to recover once they were relocated, which may indicate that the original wetland was poor brood-rearing habitat, though the results of invertebrate sampling at this site were inconclusive. This action effectively removed this brood from our study.

Growth and behavior of mallard ducklings released onto study wetlands-block 3, July 1992: Mean daily masses for broods were nearly equal through 6 days post-treatment (reference $\bar{x}$ = 61.9 and 74.8 g, treatment $\bar{x}$ = 72.3 and 59.4 g), at which point 1 brood on a treated wetland remained consistently lighter than all other broods. This difference was significant when tested at post-spray day 15 (t = -5.09, 22 df, P = 0.00004). Mortality of 4 birds occurred in this brood. These birds all lost mass prior to their death and 3 carcasses were recovered with no apparent signs of injury. We believe that their deaths were attributable to the effect of the insecticide application. Mean brood masses for block 3 at 15 days post-treatment were significantly different for birds reared on treated (n = 11, $\bar{x}$ = 117.7 g) versus reference wetlands (n = 13, $\bar{x}$ = 176.3 g) (t = -3.58, 22 df, P = 0.0017). Duckling survival at 15 days post-treatment for block 3 was higher for birds reared on the reference wetland (54%) than for birds reared on the 2 treated sites (36%) (χ^2 = 1.94, 1 df, P = 0.16). In block 3, 34 birds were lost and never found and 3 decapitated carcasses were recovered. These losses are likely attributable to mink depredation. Observations of gut contents obtained from 1 bird from each of the treated wetlands on the final day of block 3 revealed that these individuals had been feeding on northern leopard frogs (*Rana pipens*).

Block 3 experimental design changes: There were no experimental design changes during block 3.

Results summary-1992:
Morphological measurements of wings and tarsi from ducklings in broods from blocks 2 and 3 were not significantly different between birds reared on reference or

Fig. 10. Mean wing length (mm) for imprinted mallard duck broods reared on treated (n = 4) versus reference (n = 3) wetlands in western MN in 1992. Wings were measured from the point of insertion of the humerus to the distal point of the terminal phalanx-digit III. Research was conducted in 2 blocks during a 2-month period and treatment (spray) was a single aerial application of Asana® XL to wetland study sites 18 June or 15 July 1992.



treated study sites (Figs. 10 and 11). The combined mean daily masses for all birds from all 3 blocks were nearly equal throughout the 15 day post-spray period, with the exception of post-spray day 5. Birds reared on reference wetlands were lighter than birds reared on treated study sites. This difference was likely influenced by block 1 reference birds, which survived severe weather but at a reduced body mass. However, birds on reference sites were, on average, heavier than those reared on treated sites at post-spray day 15 (Fig. 12). This difference, though slight, is important biologically as mass is known be an important factor in determining a bird's ability to thermoregulate and potentially may influence survival.

The trend of duckling survival was similar for all birds, in every block, both treated and reference from -1 to 5 days post-treatment. However from 5 to 15 days post-treatment, we observed consistently lower survival for broods reared on treated sites (Fig. 13). The mean percent survival for all broods on post-spray day 15 reared on reference wetland sites ($\bar{x}$ = 65.6%, n = 5) was significantly different from mean percent survival for broods reared on treated study sites ($\bar{x}$ = 40.6%, n = 6) (t = 3.83, 8 df, P = 0.0046).

Difficulties that arose throughout the course of this study and which resulted in supplemental feeding, likely decreased the power of statistical analyses employed to detect differences in morphology, mass, and survival. Despite the difficulties encountered, statistically significant differences in duckling survival were detected.

Fig. 11. Mean tarsus length (mm) for imprinted mallard duck broods reared on treated (n = 4) versus reference (n = 3) wetlands in western MN in 1992. Measurements were from the proximal to distal portion of the tarsus. Research was conducted in 2 blocks during a 2-month period and treatment (spray) was a single aerial application of Asana® XL to wetland study sites 18 June or 15 July 1992.

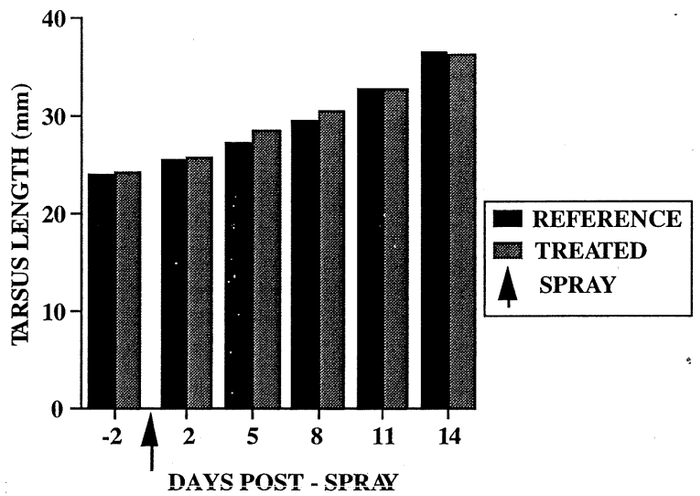


Fig. 12. Mean daily mass change for imprinted mallard duck broods reared on treated (n = 6) versus reference (n = 5) wetlands in western MN in 1992. Research was conducted in 3 blocks during a 3-month period and treatment (spray) was a single aerial application of Asana® XL to wetland study sites on 20 May, 18 June, or 15 July 1992.

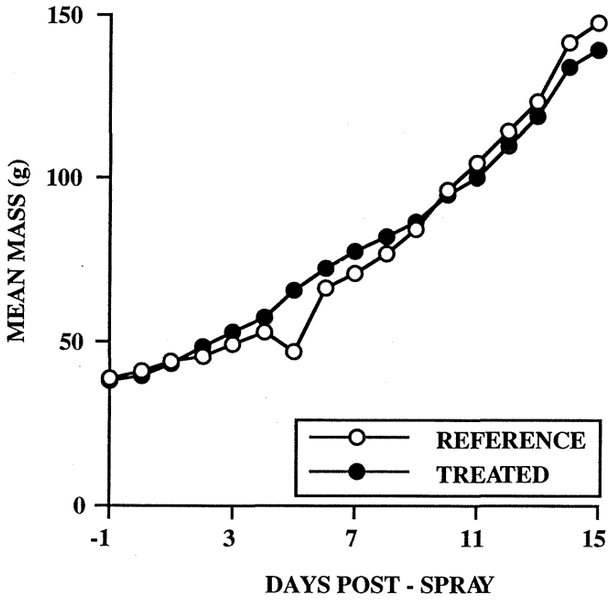
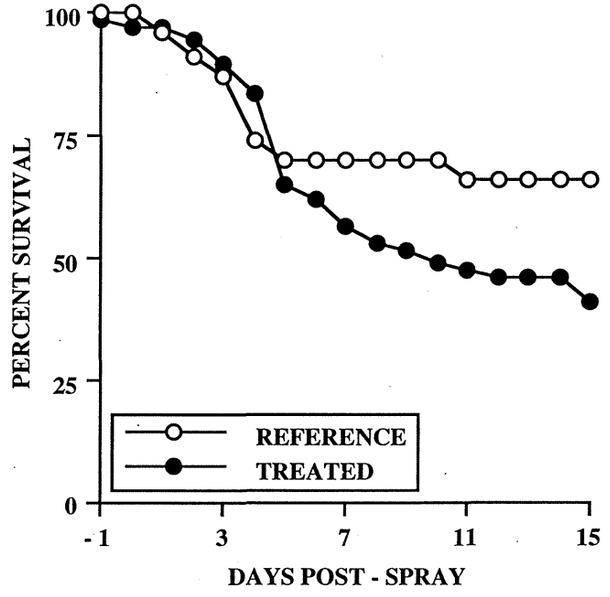


Fig. 13. Percent total survival for imprinted mallard duck broods reared on treated (n = 6) versus reference (n = 5) wetlands located in western MN in 1992. Research was conducted in 3 blocks during a 3-month period and treatment (spray) was a single aerial application of Asana® XL to wetland study sites on 20 May, 28 June, or 15 July 1992.



A.5.2. Status (uplands):

A.5.2.1. Protocol Development: Final protocol for the upland portion of the 1991 field research was developed by May 1991 and submitted for review to the Group Leader of the ~~Farmland Wildlife Research and Populations~~ FWPR group of the MN DNR at Madelia. This protocol included a review of pertinent literature and a detailed description of field work proposed for 1991.

Experimental design and protocol for the 1992 field season were finalized in April 1992. The direct effect of ~~esfenvalerate~~ Asana®XL on the insect food of ring-necked pheasant chicks, and any subsequent indirect effect on the pheasants themselves, were evaluated using imprinted pheasant chicks on upland areas, most of which were associated with wetland sites included in the wetland portion of this project. Research design and protocol were reviewed internally at the ~~University of Minnesota~~ U of MN, and through formal presentation to the ~~Farmland Wildlife Research and Populations~~ FWPR group of the MN DNR.

A.5.2.2. Study Site: Field research in 1991 was conducted at the Madelia Research Station of the MN DNR. ~~Holding pens were cleaned and repaired~~ Beginning in April, holding pens for ~~in preparation for insecticide trials conducted using~~ gray partridge and ring-necked pheasants were cleaned and repaired ~~in July~~. Field plots were laid out and buffer strips between plots were mowed in early June (see Fig. 1).

In 1992, study sites were located on ~~U. S. Fish and Wildlife Service Waterfowl Production Areas~~ USFWS WPA's in the ~~Minnesota Waterfowl and Wetlands Management Complex~~ MWWMC. These sites were dominated by native warm-season grasses. Four upland areas, approximately 0.5 ha in size, were selected for the first portion of the 1992 study. These sites were near wetland areas used simultaneously by the wetland portion of this project and 2 were treated with Asana® XL and 2 were used as reference upland sites. The second portion of the study involved 2 WPA's with two 2-ha plots on each ~~site~~ WPA. One plot on each site served as a reference upland and the other as a treated upland. Because of poor weather conditions, the first portion of the 1992 field season was treated as a pilot study. Data from this portion of the study were used to refine the protocol for subsequent field experiments and are not incorporated into this report.

A.5.2.3. Insecticides: In 1991, Asana® XL, Furadan, and Malathion were applied to 1 plot in each of 5 blocks on 22 June, and to 1 plot in each of 10 blocks on 15 and 16 July (see Fig. 1). On 15 and 16 July, plots that had been sprayed with insecticide on 22 June were sprayed a second time with the same insecticide. Asana® XL was applied at ~~0.02 lb~~ 23 g ai/ha ~~active ingredient (ai) acre (ae)~~, Malathion at ~~0.25 lb~~ 282 g ai/ha ae, and Furadan at ~~0.58 lb~~ 655 g ai/ha ae.

In 1992, Asana® XL was applied aerially on 18 June to the 2 treated ~~uplands~~ study used in the first part of the study. The 2 treated upland areas in the final portion of the study were treated on 22 July. Asana® XL was applied at the nominal rate of 34 g ~~active ingredient ha^{-1}~~ ai/ha, the maximum label rate for grasshopper control on non-crop lands (Anonymous 1991), on treated upland areas for both portions of the study.

A.5.2.4. Experimental Design: Design of the field experiment conducted in 1991 was modified slightly after review of pertinent literature and for statistical and logistical considerations (Fig. 1). Two groups of 5 blocks (5 plots in each plot) were created in early June and separated by a grassy buffer zone.

In 1992, F_1 wild stock ring-necked pheasant chicks (obtained from the Wisconsin DNR) were imprinted on humans and allowed to forage on treated and untreated (reference) uplands. Data collected on foraging chicks included mass measurements, pecking rates, and types of food consumed. There were 4 groups of birds used for each ~~part~~ portion of the study. Two ~~groups~~ broods of 10 birds were assigned to spend time on treated or reference plots exclusively. Two additional groups of 5 birds were randomly alternated between treated and reference plots once daily. Birds were approximately 3 weeks old at the conclusion of each study period. Two sets of 3 5-sweep samples were collected on each plot daily to measure insect abundance and dry mass.

A.5.2.5. Follow Up Studies: Plans and protocol were developed for the 1992 field season to expand upon research conducted in 1991. In conjunction with the wetland portion of this project, we compared growth and feeding rates of imprinted pheasant broods in upland habitats associated with wetlands (see above).

A.5.2.6. Results and Discussion:

Insecticides: In 1991, 1 tank mix sample for each insecticide was collected before each spray application, and 1 retain sample was collected from each of the insecticide containers after mixing. Circular filter papers (spray deposition cards) were placed on plots to be sprayed with insecticide and collected after they dried. Results from the analysis of tank mix, spray deposition cards, and retain samples

Table 2. Tank mix, retain, and spray deposition card analysis for insecticide active ingredients from the 1991 and 1992 field seasons of the upland portion of this study.

Application Date	Insecticide	Tank Mix		Retain		Spray Deposition Card	
		Target Rate	Lab Analyzed Rate	Target Rate	Lab Analyzed Rate	Target Rate	Lab Analyzed Rate
		(ppm)		(ppm)		(ppb)	
June 1991							
Blocks 1-5	Asana®XL	120	18	79100	83000	14	6
	Furadan	3475	240	479000	5000	414	1
	Malathion	1498	1000	599100	810000	178	107
July 1991							
Blocks 1-5	Asana®XL	120	88	79100	88000	14	7
	Furadan	3475	1220	479000	185000	414	71
	Malathion	1498	1700	599100	1020000	178	82
Blocks 6-10	Asana®XL	120	88	79100	88000	14	6
	Furadan	3475	1220	479000	185000	414	61
	Malathion	1498	1700	599100	1020000	178	82
July 1992							
Site 1	Asana®XL	----	----	----	----	13	11
Site 2	Asana®XL	----	----	----	----	13	13

confirm that the active ingredients from the insecticides were present in all 3 matrices (Table 2).

Circular filter papers were again used in 1992 to verify deposition of the active ingredient on the treated sites. Chemical analyses of these spray deposition cards also documented deposition of the active ingredient at all treated sites (Table 2).

Direct Impacts on Birds: In 1991, birds were placed on plots in wire cages, sprayed on 15 and 16 July, and subsequently placed in holding pens until death or until they were euthanized (up to 14 days post-exposure to insecticides). Following spraying, birds were observed in holding pens for obvious behavioral signs of exposure to carbamate, organophosphate, or pyrethroid insecticides (e.g., dysfunction of motor ability). Ring-necked pheasants and gray partridge exposed to insecticides did not exhibit any obvious behavioral abnormalities that can occur with exposure to pesticides. Additionally, we attributed the few deaths that did occur after spraying to injury, heat stress, and/or illness. Heads of all birds were frozen and brain tissue was analyzed from a random sample to determine brain cholinesterase activity (Table 3).

Table 3. ChE activity in a random sample of euthanized birds from the 1991 field season. Birds were euthanized 1 day after spraying. Treatments are Water (W), Malathion (M), Furadan (F), and Asana®XL (A). P-values are from ANOVA.

	ChE activity (µg/mole/min)					
	Gray partridge age class			Ring-necked pheasant age class		
Treatment	2 wk	6 wk	Adult	2 wk	6 wk	Adult
W	26.32	20.92	18.35	19.97	20.31	20.78
M	23.06	21.40	19.95	18.90	18.25	15.11
F	21.21	23.52	21.27	18.73	18.42	17.11
A	23.23	22.50	21.22	18.87	18.24	17.32
P-value	0.460	0.788	0.551	0.491	0.556	0.076

Effects of Insecticides on Insects (Indirect Effects): In 1991, insects were collected with sticky and pitfall traps until 6 August, while sweep netting continued until 27 August. Insects on sticky traps were counted in the field and identified to Order. Insects collected by sweep netting were identified to Family and/or Order, and placed in size classes within these groups, depending on the ease of identification and the relative importance in the diet of upland game bird young. Pitfall traps were not analyzed because the insects collected in these traps did not make up a significant portion of the diet of upland game bird young.

Invertebrates captured on sticky traps did not appear to be affected by insecticide applications for the 1991 field season (Fig. 14). For analysis, the 3 insecticide treatments (Malathion, Furadan, and Asana® XL) were grouped together, as were the 2 control treatments (water and untreated). Lack of a treatment effect may have occurred because of the type of insect (small and mobile) that sticky traps were able to measure on our 1991 study site can rapidly recolonize treated areas.

Invertebrates from sweep net samples were identified to Family and/or Order, oven dried, and weighed to determine biomass. For analysis, the 3 insecticide treatments

(Malathion, Furadan, and Asana® XL) were grouped together, as were the 2 control treatments (water and untreated). The average number of invertebrates per sweep sample appeared to be impacted by insecticide application (Fig. 15). Invertebrate dry mass also appeared to have been impacted by insecticide application (Fig. 16). Areas receiving multiple insecticide treatments may show even greater invertebrate biomass reductions when compared to areas receiving only a single treatment (Fig. 16).

In 1992, sweep net samples were collected each day at sites that were used by imprinted birds. Invertebrates in these samples were identified to Family and/or Order, and were dried prior to determining mass. Invertebrates (total numbers per sweep net sample) on both study sites appear to have been reduced by the insecticide application (Fig. 17). Invertebrate dry mass on both sites also appears to have been impacted after insecticide treatment (Fig. 18).

Although both invertebrate numbers and dry mass were reduced on both study sites, ring-necked pheasant chicks did not appear to respond to these changes (Fig. 19). Birds feeding on the treated area on site 1 generally had a lower average mass change than those birds feeding on the untreated area on Site 1 (Fig. 19), and was observed both before and after insecticide application. However, this relationship was reversed for birds feeding on site 2: the birds feeding on the treated areas generally had a greater average mass change compared to birds feeding on the untreated area (Fig. 19).

Feeding rates of the pheasant chicks on site 1 were greater for those birds feeding on the reference area than on the treated area (Fig. 20). Those birds feeding on the reference area also gained more mass than the birds feeding on the treated area. However, birds feeding on the treated area of site 2 gained more mass than birds feeding on the reference area, even though there was little difference in their feeding rates (Fig. 20).

We believe there are 2 possible explanations for the lack of response. First, initial differences in the invertebrate populations between sites, and between plots within sites, may have continued after the insecticide application. A minimum "threshold" in invertebrate numbers and/or biomass necessary to result in a measurable effect in the pheasant chicks may not have been reached.

The second explanation is that recolonization of the treated plots by mobile invertebrates occurred rapidly, even though the insecticide kept the invertebrate populations depressed. In this study, ring-necked pheasant chicks were observed consuming dead invertebrates, and this could account for the apparent lack of response

Fig. 14. Average number of invertebrates collected on sticky traps on study plots at the MN DNR FWPR facility in 1991. All insecticide-treated plots were pooled (treated), as were water and no-treatment plots (untreated).

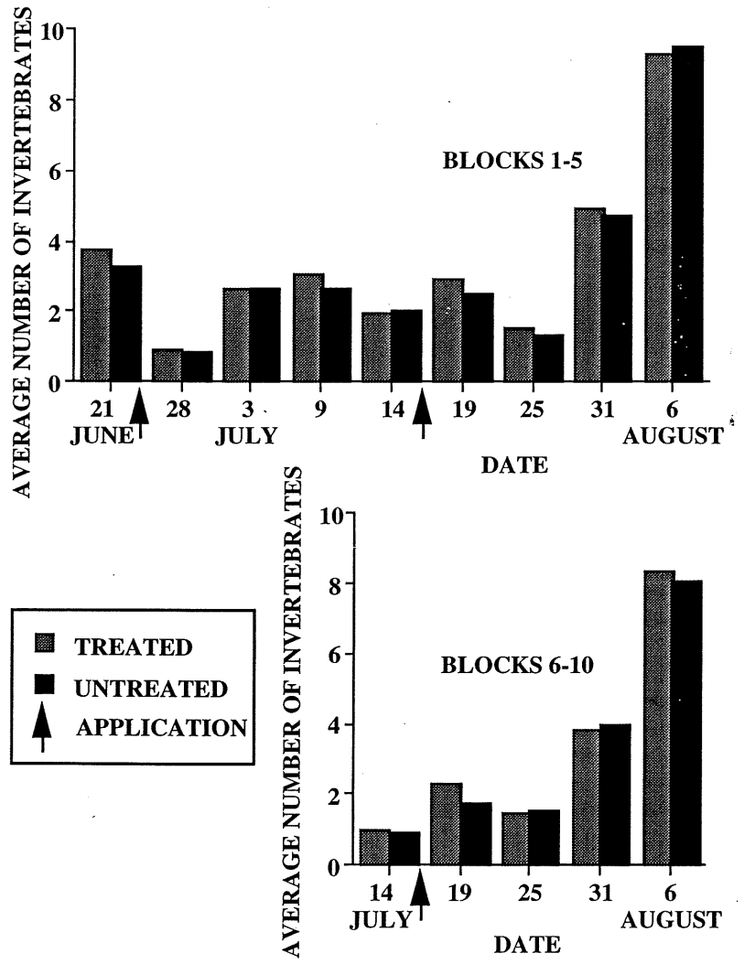


Fig. 15. Average number of invertebrates collected per sweep-net sample on study plots at the MN DNR FWPR facility in 1991. All insecticide-treated plots were pooled (treated), as were water and no-treatment plots (untreated).

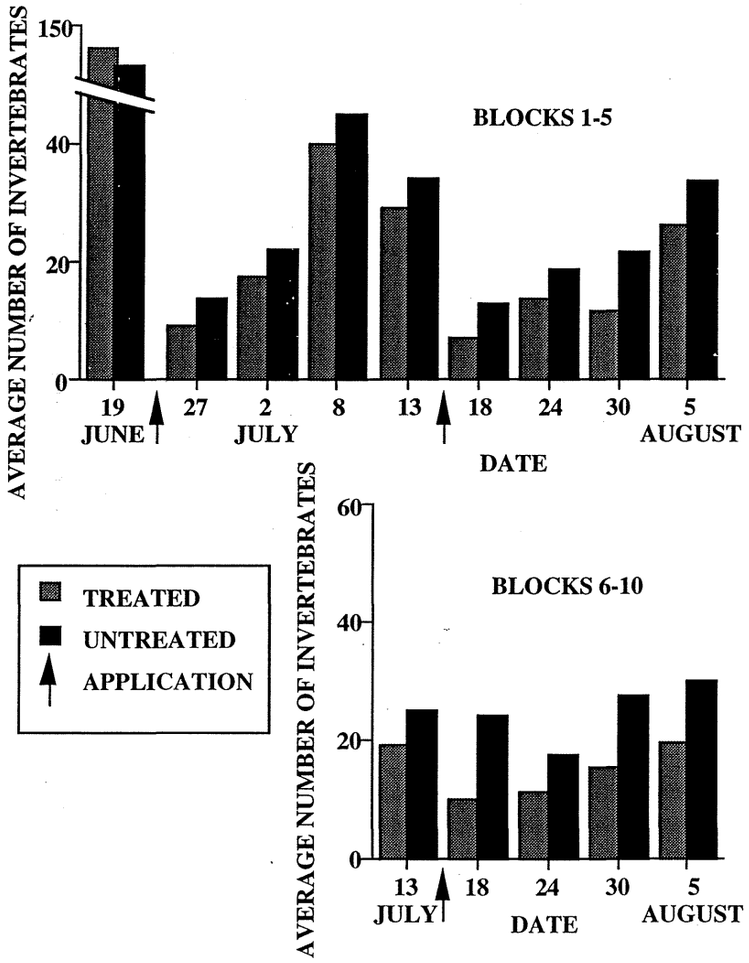
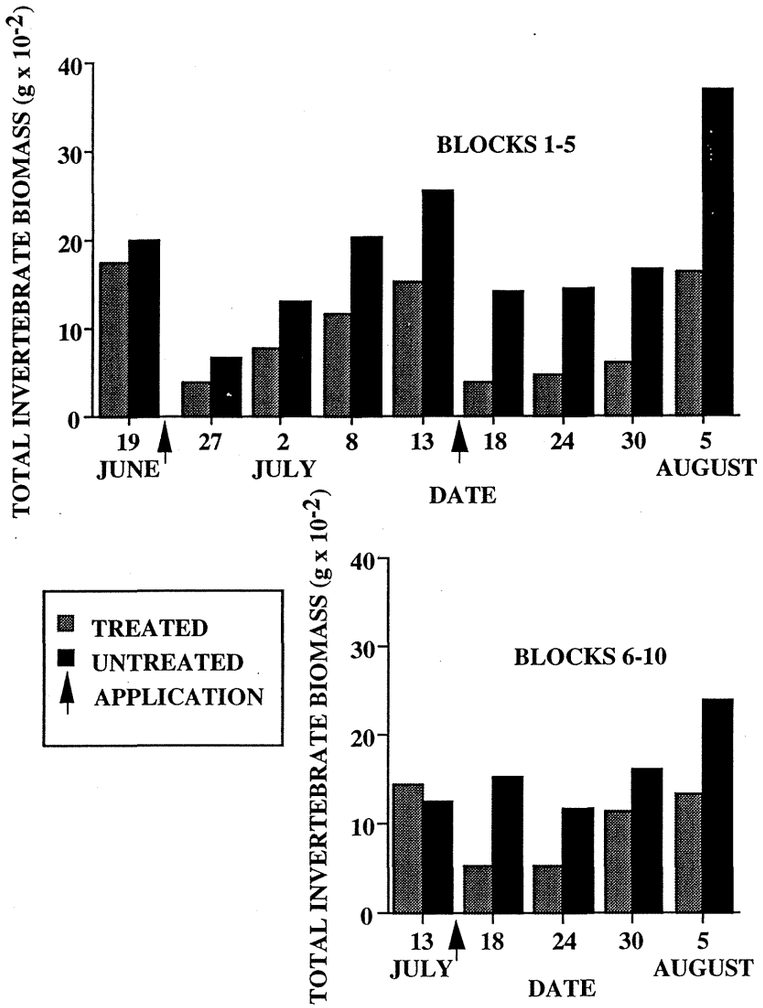


Fig. 16. Biomass of invertebrates collected in sweep nets on study plots at the MN DNR FWPR facility in 1991. All insecticide-treated plots were pooled (treated), as were water and no-treatment plots (untreated).



of the birds to the changes in living invertebrate abundance caused by the insecticide application.

In this study, insecticide applications to habitat utilized by upland game bird chicks had no significant effect on growth of imprinted pheasant chicks. Our 1992 study consisted of 1 application of Asana® XL at 34 g ai/ha on upland habitat areas approximately 2 ha in size surrounded by similar, untreated habitat. However, the results obtained in this study should be extended to other situations with caution. Multiple insecticide applications, treated areas that are significantly larger than those in this study, or insecticide applications to habitats that may not be recolonized as rapidly as those in this study (e.g., roadsides adjacent to row crops) may make significant indirect effects of insecticide applications on insect-dependent birds more likely.

Fig. 17. Average invertebrate numbers per sweep-net sample from reference and treated plots on 1992 study sites in western MN. Invertebrates were sampled up to 6 times daily on each plot with a sweep net. Timing of insecticide application is indicated by an arrow.

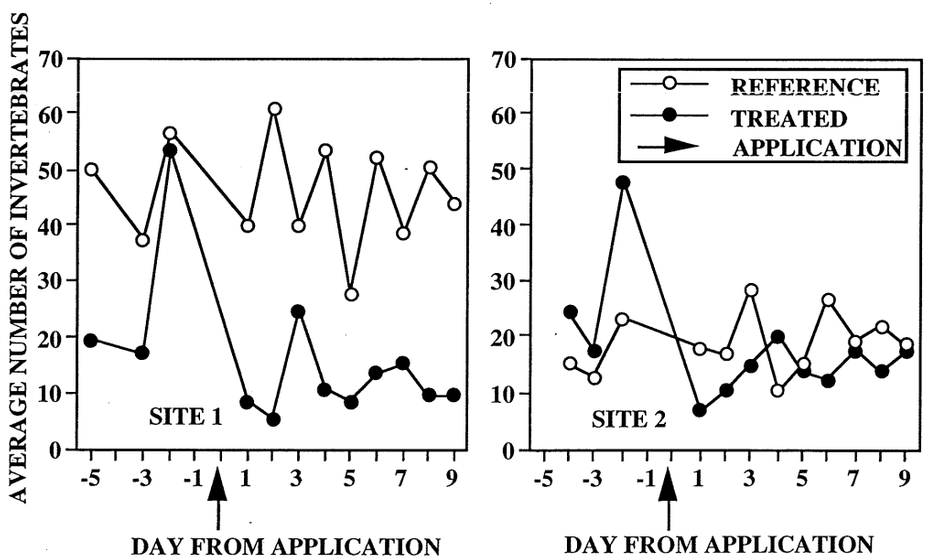


Fig. 18. Biomass of invertebrates collected in sweep nets from 1992 study sites in western MN. All invertebrates were oven-dried and weighed to determine dry mass. Timing of insecticide application is indicated by an arrow.

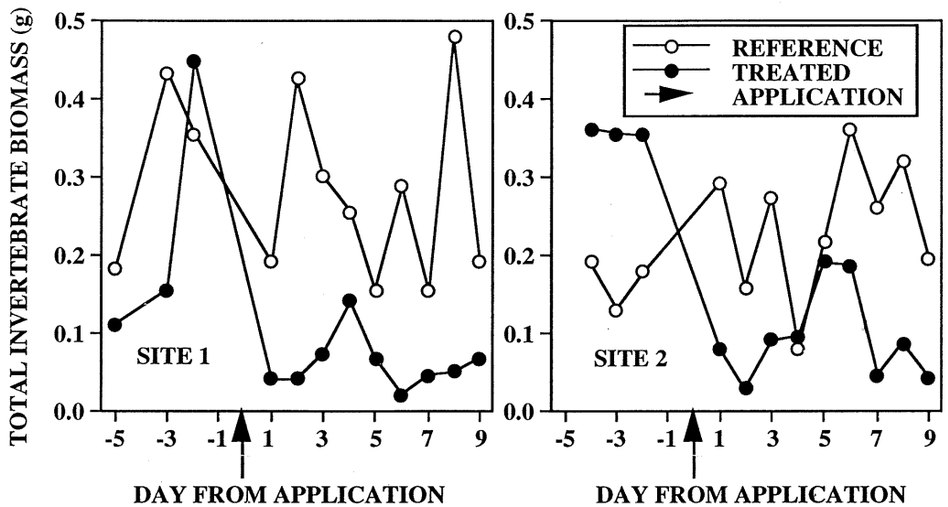


Fig. 19. Average daily mass change for ring-necked pheasant chicks allowed to forage on reference or untreated upland plots in western MN in 1992. Positive values indicate an increase in mass, while negative values indicate mass loss.

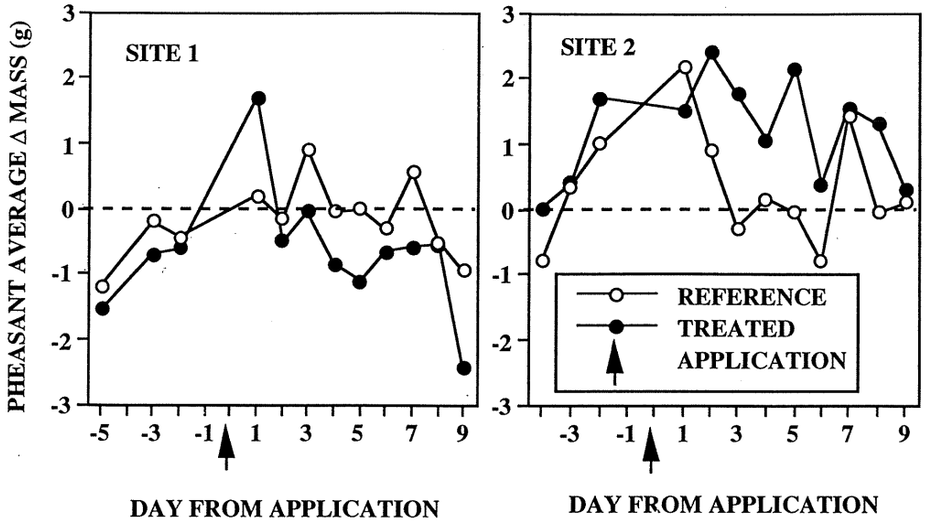
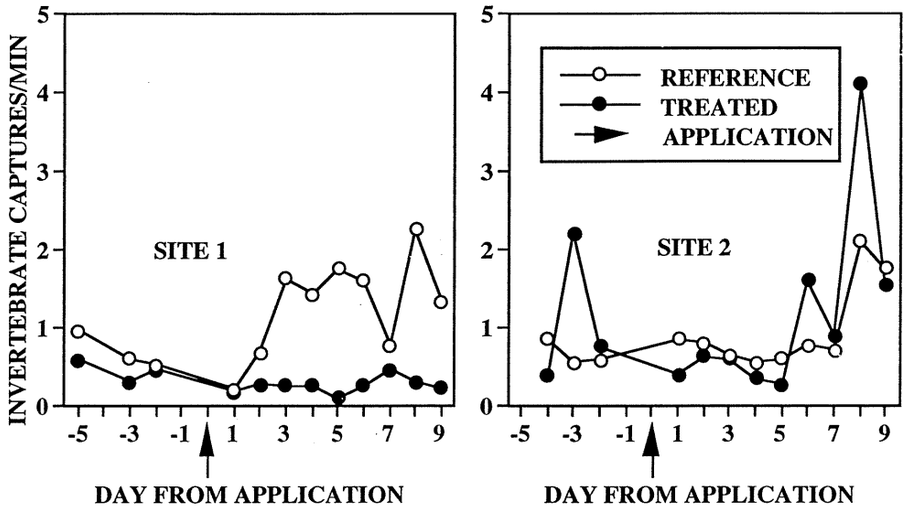


Fig. 20. Average feeding rate as measured by invertebrate captures per minute feeding for pheasant chicks allowed to forage on treated or untreated upland areas in western MN in 1992. Timing of insecticide application is indicated by an arrow.



A.6. Benefits (wetlands and uplands): Initial studies of insecticide effects suggest a high potential to reduce foods of breeding birds and their young or to cause direct mortality of birds. Understanding effects of these chemicals will help develop techniques to minimize negative impacts on wildlife. By incorporating information on insecticide effects, this research will improve the implementation of existing wildlife management plans while aiding farmers in planning frequency of application, chemical selection and method of application.

IV. EVALUATION:

A. Wetlands: Recent studies completed in ~~North Dakota~~ ND (Grue et al. 1989) documented direct and indirect mortality on waterfowl and invertebrates from spraying agricultural insecticides on wetlands. The threat of grasshopper outbreaks, as well as

tent caterpillars, indicates the necessity of evaluating the potential of a similar problem in ~~Minnesota~~ MN. The results of this project will be evaluated by 1) determining if direct and indirect effects on waterfowl and their food base occurs; 2) determining the extent of chemical contamination of a wetland through routine chemical application; 3) assessing procedural modifications in operations that can be suggested to minimize impacts of agricultural pest control agents on wetland wildlife while maximizing pest control for crop harvest maximization.

B. Uplands: For the FY92-93 biennium this program can be evaluated by its ability to 1) assess the direct impact of selected agricultural insecticides on upland game birds, 2) assess indirect effects of selected agricultural insecticides on upland game birds through impacts on invertebrate foods, and 3) provide sound scientific data to resource manager, legislators, and producers concerning application of agricultural insecticides in ~~Minnesota~~ MN.

In the long term, evaluation of this project's success will be in the development of procedures and practices for prescribing and applying agricultural chemicals that minimize potential negative environmental impacts. Concern for the environment as a whole needs to be incorporated into agricultural production practices based on the best available information.

V. CONTEXT

A. Wetlands:

1. Much effort by public and private agencies has been devoted to protecting existing wetlands and restoring wetlands lost because of encroachment from human activities. Little effort, however, has been directed towards understanding how anthropogenic impacts may degrade the quality of prairie wetlands. An increase in our understanding of how to minimize negative impacts on wetlands ecosystems will aid in maintaining and improving remaining habitat. The information generated by this project will be used by wetland managers, farmers, agricultural extension personnel, and state and federal agencies in seeking solutions to providing quality prairie wetland habitat for both humans and wildlife.

2. Recent studies completed in ~~North Dakota~~ ND by the ~~U.S. Fish and Wildlife Service~~ USFWS documented direct mortality on waterfowl and reduced invertebrate populations caused by the spraying of an insecticide on wetlands. Controlled field experiments will be conducted to further assess the sublethal impacts of agricultural insecticides on waterfowl and their invertebrate food base.

3. Not applicable.

4. Biennial Budget System Program Title and Budget: Impact of insecticides--AID359232.

B. Uplands:

1. Resource professionals and agricultural producers alike are concerned about the potential negative environmental impacts of chemical application in agricultural production. Sustainable agricultural practices must efficiently use resources while minimizing potential environmental degradation. This project will establish important information concerning potential negative impacts associated with chemical pesticide applications that can be used to make informed application and policy decision. This project also brings together experts from state (~~Minnesota~~ MN DNR) and federal (~~U.S. Fish and Wildlife Service~~ USFWS) agencies and academic institutions (~~University of Minnesota~~ U of MN) to address topics of mutual state and national concern.

2. Agricultural pesticides are suspected of impacting wild bird populations both directly, through increased mortality rates, and indirectly through impacts on food resources. Direct mortality is generally thought to be related to toxicity, as measured by lethal exposure (e.g., Smith 1987), although documenting reduced survival in wild bird populations as a result of exposure to insecticides is difficult. However, field evidence suggests that insecticides, including organophosphates and carbamates, can cause direct mortality in birds (e.g., White et al. 1982, Henny et al. 1987, Blus et al. 1989) and may increase susceptibility to predation. Pesticides can also impact bird populations indirectly through effects on insect prey, as insects constitute the majority of the diet of many juvenile birds, including young gray partridge (Southwood and Cross 1969, Potts 1986) and pheasants (Hill 1985). Broad-spectrum insecticides have the potential to directly reduce insect availability (Potts 1986, Rands 1986, Rands et al. 1988) and herbicides can indirectly affect insect availability by killing host plants important to insect food of chicks (e.g., Southwood and Cross 1969, Vickerman 1974, Sotherton 1982).

Both direct and indirect impacts of agricultural insecticides on game birds have generally been measured indirectly by estimating productivity or density in areas under different insecticide application regimes (e.g., Sotherton and Robertson 1990). Often, chemical application is controlled, while initial bird densities are unknown. Studies evaluating the effect of currently used insecticides in field settings where both chemical application and bird exposure are controlled are generally lacking. Recently, the usefulness of controlling both chemical application and bird density has been demonstrated by Grue et al. (1989) with waterfowl.

Previous field studies of direct and indirect effects of pesticide application related to agricultural production conducted in the western U.S. and Canada have been equivocal in their assessment of impact on vertebrates. In general, these studies have lacked replication and have been hampered by unanticipated environmental variability. We propose to conduct an experimental study designed to reflect field conditions in MN, that allows us to draw statistically valid inference from our results. We propose to do this by controlling both insecticide application and exposure in the subject birds, and monitoring the direct and potential indirect effects on game birds.

3. Prior research documenting the potential impacts of agricultural chemicals on wetland and upland wildlife in ~~Minnesota~~ MN has not been funded by the LCMR. Results of related research will be incorporated as appropriate through published and unpublished literature. The intent of this project is to provide sound information on which to base decisions concerning the potential impacts of chemical application on ~~Minnesota~~ MN wildlife. Additional funding beyond the FY92-93 biennium may be sought from the LCMR, based upon the results of this project and future agricultural practices in the state.

4. Not applicable

5. Biennial Budget System Program Title and Budget: Impact of insecticides--AID359232.

VI. QUALIFICATIONS

1. Program Manager:

Dr. Alfred H. Berner
Group Leader
Farmland Wildlife Populations and Research
Section of Wildlife Populations and Research Unit
Minnesota Department of Natural Resources

Adjunct Professor
Department of Fisheries and Wildlife
University of Minnesota

Ph.D. Wildlife Ecology, Michigan State University, 1969
M.S. Wildlife Ecology, Michigan State University, 1965

Dr. Berner has conducted research and written on a wide variety of subjects but is best known for his work on the impacts of federal farm programs on land use and pheasant populations. He also has experience in refereeing journal articles particularly in the areas of pheasant ecology and management, and impacts of land use on pheasant populations. Dr. Berner's primary role will be to act as Program Manager, and provide advisory and logistic support to the upland portion of this program.

2. Major Cooperators:

A. Dr. David E. Andersen (Uplands)
Assistant Unit Leader - Wildlife
Minnesota Cooperative Fish and Wildlife Research Unit
U.S. Fish and Wildlife Service

Assistant Professor
Department of Fisheries and Wildlife
University of Minnesota

Ph.D. Wildlife Ecology, Zoology, University of Wisconsin, 1988
M.S. Wildlife Ecology, University of Wisconsin, 1984

Dr. Andersen has conducted research on the impacts of human activity on wild birds and other wildlife. His experience includes writing, refereeing, and editing publications in avian ecology and management and working with government agencies concerning mitigation of negative human impacts. Dr. Andersen's primary role will be to conduct the upland portion of the project jointly with Dr. Berner and advising the graduate student who will conduct the upland portion of the project as a Master's project.

B. Dr. Mary G. Henry (Wetlands)
Unit Leader
Minnesota Cooperative Fish and Wildlife Research Unit
U.S. Fish and Wildlife Service

Associate Professor
Department of Fisheries and Wildlife
University of Minnesota

Ph.D. Animal Ecology, Iowa State University, 1984
M.S. Environmental Health, Purdue University, 1978

Dr. Henry has worked extensively in the environmental contaminants field for the past fifteen years. Her experience encompasses bioassessment of contaminant effects on aquatic systems affected by acid precipitation, agricultural insecticides, PCBs, and polycyclic aromatic hydrocarbons. Dr. Henry has conducted this work in association with state and federal agencies and university staff. Her role will be to coordinate and advise graduate students investigating the wetland impacts portion of this project. Dr. Tome will assist in advising graduate students and participate on their committees.

C. Dr. Michael C. Zicus
Wetland Wildlife Populations and Research Group
MN Department of Natural Resources

Ph.D. Wildlife Management, University of MN, 1976
M.S. Wildlife Management, University of MN, 1974

Dr. Zicus has been a waterfowl biologist with the DNR since 1978. He has conducted research and published findings on Canada geese and cavity nesting waterfowl. Since 1985, he also has been a member of the Metropolitan Mosquito Control District's Scientific Peer Review Panel which has advised the district on research regarding the effects of mosquito control on non-target organisms. Dr. Zicus will help coordinate necessary field aspects of the project with DNR wildlife managers.

D. Dr. Michael W. Tome
Wildlife Biologist
NAWWO
U.S. Fish and Wildlife Service
340 Arlington Square
Washington, DC 20240

Ph.D. Natural Resource Management, The University of Michigan, 1986
M.S. Wildlife Management, University of Maine, 1982

Dr. Tome has conducted research on waterfowl foraging ecology and energetics, and the impacts of agricultural pesticides on waterfowl and wetlands. Dr. Tome has worked extensively with resource agencies and agriculture extension personnel in identifying agricultural practices that will meet the needs of the farmer while maintaining the quality of wetland habitats. He has written, edited and refereed publications concerning waterfowl ecology and the impacts of contaminants on wildlife. Dr. Tome's primary role will be to assist in coordinating and advising graduate students on the wetland portion of the project with Dr. Henry and serve on the committee of the graduate students who will conduct this portion of the project for Master's degrees.

VII. REPORTING REQUIREMENTS

Semiannual status reports will be submitted not later than January 1, 1992, July 1, 1992, January 1, 1993 and a final status report by June 30 1993.

VIII. LITERATURE CITED

Anonymous. 1991. 1991 Crop protection Chemicals Reference. Seventh edition. John Wiley and Sons, New York and Chemical and Pharmaceutical Publ. Corp., Paris.

Blus, L. J., C. S. Staley, C. J. Henny, G. W. Pendleton, T. H. Craig, E. H. Craig, and D. K. Halford. 1989. Effects of organophosphorus insecticides on sage grouse in southeastern Idaho. J. Wildl. Manage. 53:1139-1146.

Chura, N. J. 1961 Food availability and preferences of juvenile mallards. Trans. N. Am. Wildl. Nat. Res. Conf. 26:121-133.

Coats, J. R., D. M. Symonik, S. P. Bradbury, S. D. Dyer, L. K. Timson, and G. J. Atchison. 1989. Toxicology of synthetic pyrethroids in aquatic organisms: an overview. Environ. Toxicol. and Chem. 8:671-679.

Cooper, J. A., C. M. Potter, and C. H. Welling. 1989. Mallard duckling growth and behavior in methoprene treated wetlands. Unpublished report by the Department of Fisheries and Wildlife, University of MN, St. Paul MN 55018 to the Legislative Commission on Minnesota Resources, State Office Building, St. Paul, MN 55155.

Cowardin, L., V. Carter, F. C. Golet, and E. T. LaRoe. 1979. Classification of wetland and deepwater habitats of the United States. U.S. Fish and Wildlife Service, Wash., D.C.

Erpelding, R., R. O. Kimmel, and D. J. Lockman. 1987. Foods and feeding behavior of young gray partridge in MN. Pages 17-30 *in* R. O. Kimmel, J. W. Schultz, and G. J. Mitchell, eds. Perdix IV: Gray partridge workshop. Minn. Dep. Nat. Resour., Madelia.

Grue, C. E., M. W. Tome, T. A. Messmer, D. B. Henry, G. A. Swanson, and L. R. DeWeese. 1989. Agricultural chemicals and prairie pothole wetlands: meeting the needs of the resource and the farmer--U.S. perspective. Trans. 54th N. A. Wildl. and Nat. Res. Conf. 43-58.

Henny, C. J., E. J. Kolbe, E. F. Hill, and L. J. Blus. 1987. Case histories of bald eagles and other raptors killed by organophosphorus insecticides topically applied to livestock. J. Wildl. Dis. 23:292-295.

Hill, D. A. 1985. The feeding ecology and survival of pheasant chicks on arable farmland. J. Appl. Ecol. 22:645-654.

Hill, D., R. Wright, and M. Street. 1987. Survival of mallard ducklings *Anas platyrhynchos* and competition with fish for invertebrates on a flooded gravel quarry in England. Ibis 129:159-167.

Hunter, M. L., J. W. Witham, and H. Dow. 1984. Effects of a carbaryl induced depression in invertebrate abundance on the growth and behavior of American black duck and mallard ducklings. Can. J. Zool. 62:452-456.

Hunter, M. L., J. J. Jones, K. E. Gibbs, and J. R. Moring. 1986. Duckling responses to lake acidification: do black ducks and fish compete? Oikos 47:26-32.

Joselyn, G. B., and G. I. Tate. 1972. Practical aspects of managing roadside cover for nesting pheasants. J. Wildl. Manage. 36:1-11.

Kimmel, R. O., and W. M. Healy. 1987. Imprinting - a technique for wildlife research. Pages 32-57 *in* R. O. Kimmel, J. W. Schultz, and G. J. Mitchell, eds. Perdix IV: Gray partridge workshop. Minn. Dep. Nat. Resour., Madelia.

Lees, P. R., and M. Street. 1974. The feeding ecology of young Mallard (*Anas platyrhynchos*) and tufted duck (*Aythya fuligula*) in wet gravel quarries. Eleventh Internat. Congress of Game Biologists.

Lokemoen, J. T., H. A. Doty, D. E. Sharp, and J. E. Neaville. 1982. Electric fences to reduce mammalian predation on waterfowl nests. Wildl. Soc. Bull. 10:318-323.

Loughrey, A. G., and R. H. Stinson. 1955. Feeding habits of juvenile ring-necked pheasants on Pelee Island, Ontario. Can. Field-Nat. 69:59-65.

Murkin, H. R., P. G. Abbott, and J. A. Kadlec. 1983. A comparison of activity traps and sweep nets for sampling nektonic invertebrates in wetlands. Freshwater Invert. Biol. 2:99-106.

Nelson, D. R., R. O. Kimmel, and M. J. Fryendall. 1990. Ring-necked pheasant and gray partridge brood habitat in roadsides and managed grasslands. Pages 103-112 *in* K. E. Church, R. E. Warner, and S. J. Brady, eds. Perdix V: Gray partridge and ring-necked pheasant workshop. Kans. Dep. Wildl. and Parks, Emporia.

Potts, G. R. 1986. The partridge: pesticides, predation and conservation. Collins, London, U.K. 274pp.

Perret, N. G. 1962. The spring and summer foods of the common mallard (*Anas platyrhynchos* L.) in south central Manitoba. M.S. Thesis Univ. of British Columbia, Vancouver.

Rands, M. R. W. 1986. The survival of gamebird chicks in relation to pesticide use on cereal. Ibis 128:57-64.

Rands, M. R. W., P. J. Hudson, and N. W. Sotherton. 1988. Gamebird ecology, conservation and agriculture. Pages 1-17 *in* P. J. Hudson, and M. R. W. Rands, eds. Ecology and management of gamebirds. Blackwell Sci. Publ. London, U.K.

Shaw, S. P., and C. G. Fredine. 1956. Wetlands of the United States. U.S. Fish Wildl. Serv., Circ. 39, Wash., D.C.

Smith, G. J. 1987. Pesticide use and toxicology in relation to wildlife: Organophosphorus and carbamate compounds. Resour. Publ. 170. U.S. Fish and Wildl. Serv., Washington, D.C. 171pp.

Sotherton, N. W. 1982. Effects of herbicides on the chrysomelid beetle *Gastrophysa polygoni* (L.) in laboratory and field. Zeitschrift fur angewandte Entomologie 94:446-451.

Sotherton, N. W., and P. A. Robertson. 1990. Indirect impacts of pesticides on the production of wild gamebirds in Britain. Pages 84-102 *in* K. E. Church, R. E. Warner, and S. J. Brady, eds. Perdix V: Gray partridge and ring-necked pheasant workshop. Kans. Dep. Wildl. and Parks, Emporia.

Southwood, T. R. E., and D. J. Cross. 1969. Ecology of the partridge. III. Breeding success and the abundance of insects in natural habitats. J. Anim. Ecol. 38:497-509.

U.S. Environmental Protection Agency. 1989. Effects, persistence, and distribution of Asana® XL in littoral enclosures. EPA-Duluth, Univ. Wis. Superior. 276pp.

Varland, K. 1985. Why roadsides for wildlife? Minn. Volunteer 2:3-8.

Vickerman, G. P. 1974. Some effects of grass weed control on the arthropod fauna of cereals. British Weed Control Conf. 12:929-939.
White, D. H., C. A. Mitchell, L. D. Wynn, E. L. Flickinger, and E. J. Kolbe. 1982. Organophosphate insecticide poisoning of Canada geese in the Texas Panhandle. J. Field Ornithol. 53:22-27.