AVIAN EXPOSURE TO AGRICULTURAL CHEMICALS IN MINNESOTA

FINAL PILOT STUDY REPORT

TO

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DIVISION OF CONTAMINANTS

FROM

MINNESOTA COOPERATIVE FISH AND WILDLIFE RESEARCH UNIT
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SUMMARY

In June 1992, we collected 52 plasma samples from nestling red-winged blackbirds *(Agelaius phoeniceus)*, house sparrows *(Passer domesticus)*, and brown-headed cowbirds *(Molothrus ater)* at 5 study sites near the town of Ada in northwestern Minnesota. Three sites were adjacent to sugar beet fields that were likely to be treated with Lorsban® (an organophosphate insecticide) for control of sugar beet root maggots *(Tetanops myopaeformis)*, and 2 sites were distant from fields likely to be treated (reference sites). Application of Lorsban® in fields surrounding study plots was monitored through contact with landowners and direct observations. Cholinesterase (ChE) activity levels [total cholinesterase (TChE), acetylcholinesterase (AChE), and butyrylcholinesterase(BChE)] in nestling plasma were measured and tested (TChE and AChE) for reactivation in the presence of 2-PAM, an indication of exposure to organophosphate insecticides. In addition, 11 nestlings were euthanized and in these samples we measured brain ChE activity and reactivation, and we analyzed gastrointestinal tracts and carcass washes for Lorsban® residues. Total ChE and BChE activity were lowest in similar-aged nestlings at sites adjacent to treated beet fields (TChE, $t = -2.51$, $d.f. = 21$, $P = 0.033$; BChE, $t = -2.56$, $d.f. = 21$, $P = 0.043$). Nestlings from sites that were near fields where Lorsban® was applied were more likely to exhibit plasma AChE reactivation than nestlings from reference sites where OP or carbamate insecticide application was improbable ($X^2 = 3.805$, $d.f. = 1$, $P \approx 0.05$). The magnitude of plasma ChE reactivation was highest within 1-3 days of insecticide application, although significant reactivation was measured up to 11 days after application of Lorsban®. Plasma AChE reactivation appeared to be a more sensitive indicator of exposure to ChE-inhibiting insecticides than absolute TChE or BChE activity levels. The occurrence of Lorsban® residue in gastrointestinal tracts and carcass washes suggested that nestlings were exposed to insecticides through prey delivery and/or physical contact with adults.

INTRODUCTION

In the United States organophosphate (OP) and carbamate insecticides are used on a wide variety of crops including cotton, white and wild rice, potato, sunflower, field corn, and winter wheat (Smith 1987, White et al. 1990, Minnesota Department of Agriculture 1990, Agricultural Statistics Board 1991). Smith (1987) estimated (from U.S. Environmental Protection Agency data) that from 1981-1987, 160 million acre-treatments of OP's and carbamates were applied annually to agricultural crops and forests in the U.S. In a survey of agricultural pesticide use in Minnesota in 1990, 85% of the acres reported treated with insecticide or miticide were reported as treated with OP's or carbamates (Minnesota Department of Agriculture 1990), approximately 1,600,000 acres -- approximately 7 % of all agricultural lands in Minnesota. Because a large total area is treated with these chemicals annually, it is likely that nontarget species are exposed to OP and carbamate insecticides and several studies indicate exposure of nontarget vertebrate species (Deeley 1980, DeWeese et al. 1983, Grue et al. 1983, McEwen et al. 1986, Hooper et al. 1989). Birds are frequently the focus of these studies because they are common in agricultural habitats and some bird species exhibit traits [such as insectivory and nesting in or adjacent to agricultural cropland (Byran and Best 1991)] that make them likely to be exposed to these compounds.

Considerable field and laboratory research has focused on the toxicity and sublethal effects of cholinesterase-inhibiting compounds to birds (Ludke et al. 1975; Grue et al. 1982; Powell 1984; Meyers et al. 1990; Martin et al. 1991*a,b*). Although, a number of avian die-offs have been attributed to exposure to these compounds (Seabloom et al. 1973, Mendelssohn and Paz 1977, Frank et al. 1991), the extent to which migratory birds are exposed to operational applications of these compounds is not well documented.

There are a large number of OP and carbamate compounds (Smith 1987) and most inhibit the enzyme cholinesterase (ChE) (O'Brien 1976). The inhibition of ChE (Fig. 1, equation 1) in birds leads to the accumulation of the neurotransmitter acetylcholine (ACh) (Fig. 1, equation 2).

In animals, this can cause a variety of effects, including loss of coordination, appetite suppression, tremors, convulsions and sometimes death, generally as a result of respiratory failure (O'Brien 1976, Hill and Camaradese 1984, Grue et al. 1991). The significance of sublethal exposure in birds is not clear, but some research indicates that survival and reproduction may be negatively affected (Galindo et al. 1985, Brewer et al. 1988, Buerger et al. 1991, Hunt et al. 1992). The direct measurement of OP and carbamate insecticide residues from operational applications is difficult and often impractical because these insecticides have short half lives in biological tissue and a large number of compounds are in use. However, measurement of ChE activity or ChE inhibition in brain tissue or plasma of birds can be used as an indicator of exposure to ChE-inhibiting insecticides (Ludke et al. 1975, Hill and Fleming 1982, Wilson et al. 1991).

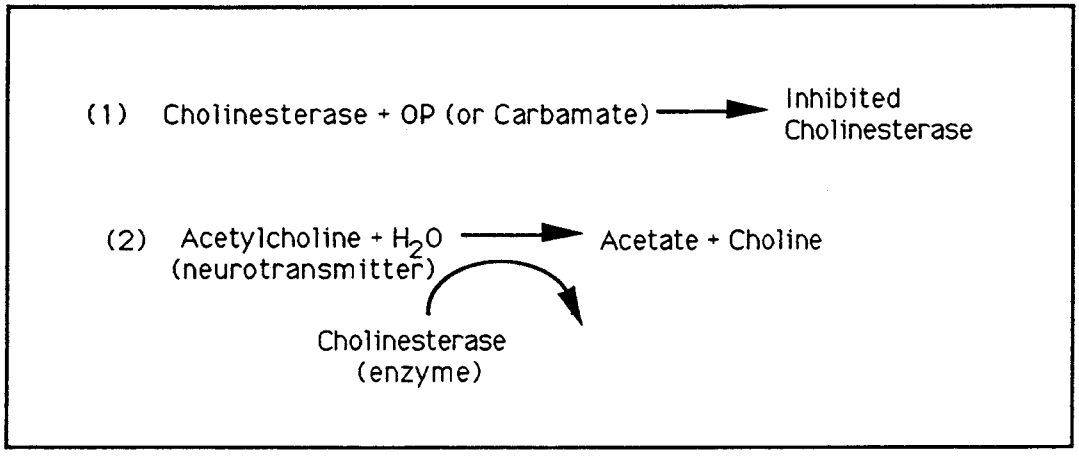

Figure 1. Cholinesterase inhibition by organophosphate (OP) or carbamate insecticides and cholinesterase catalyzed reaction of acetylcholine.

We used ChE activity measurements (Ellman et al. 1961, Hill and Fleming 1982) and ChE reactivation techniques (Martin et al. 1981, Hooper et al. 1989, Wilson et al. 1991) to evaluate the exposure of nestling birds to Lorsban® (an OP insecticide) applied to sugar beet fields in northwestern Minnesota. Lorsban® is a trade name for chlorpyrifos (phosphorothioic

acid 0,0, - diethyl 0 - (3,5,6 - trichloro - 2 - pyrdinyl) esther). We collected blood samples from red-winged blackbird (*Agelaius phoeniceus*), house sparrow (*Passer domesticus*), and brown-headed cowbird (*Molothrus ater*) nestlings from nests near sugar beet fields. Our goal was to evaluate the feasibility of using plasma and brain ChE activity and reactivation as a means of assessing exposure of nontarget organisms to the operational use of ChE-inhibiting insecticides in an agricultural setting (McInnes and Andersen 1992).

STUDY SITES

Site Selection

We selected Norman County, in northwestern Minnesota (Fig. 2), as the location for this pilot field study because of the cooperation and recommendation of a local crop consultant (D. Berglund) who indicated that there was a high probability of a sugar beet root maggot (*Tetanops myopaeformis*) (Rder.) outbreak in this area in 1992 and that the OP insecticide Lorsban® is commonly applied for the control of this pest. In addition, the crop consultant provided us with information on the timing of the maggot outbreak and the probable timing of Lorsban® application and referred us to landowners who raised sugar beets in the area. We considered working in other locations in Minnesota dominated by different agricultural crops, but found that the use of OP's and carbamates were less predictable or the timing was inappropriate for working with nestlings. All field sites were located in Norman County, within 18 km of the town of Ada (Fig. 3). These sites were on private land with the exception of a wetland sampled as a reference site. We identified possible study sites through evaluation of maps of the area and by driving local roads searching for wetland areas. We selected sites close to sugar beet fields where red-winged blackbird nests were present.

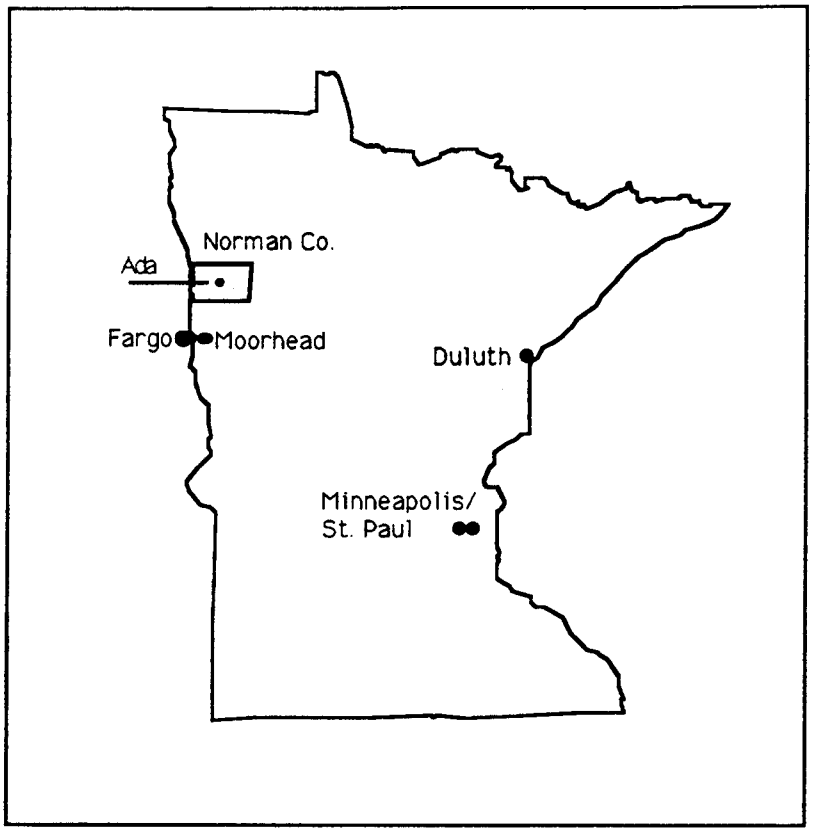

Figure 2. Location of the study area in northwestern Minnesota.

DNR Wetland

This wetland was located on a 64 ha wildlife area managed by the Minnesota Department of Natural Resources (DNR) in Section 12 of Lake Ida Township (Fig. 3). The wetland comprised the majority of the site, but was bordered by some areas of shrubby and grassy vegetation. Agricultural land near this site included grass and hay fields, fallow land with minimal vegetation (set aside), and wheat fields. There were no beet fields or other crops commonly treated with OP's or carbamates adjacent to or within 300 m of this wetland. We located and visited 25 red-winged blackbird nests at the southern end of the wetland.

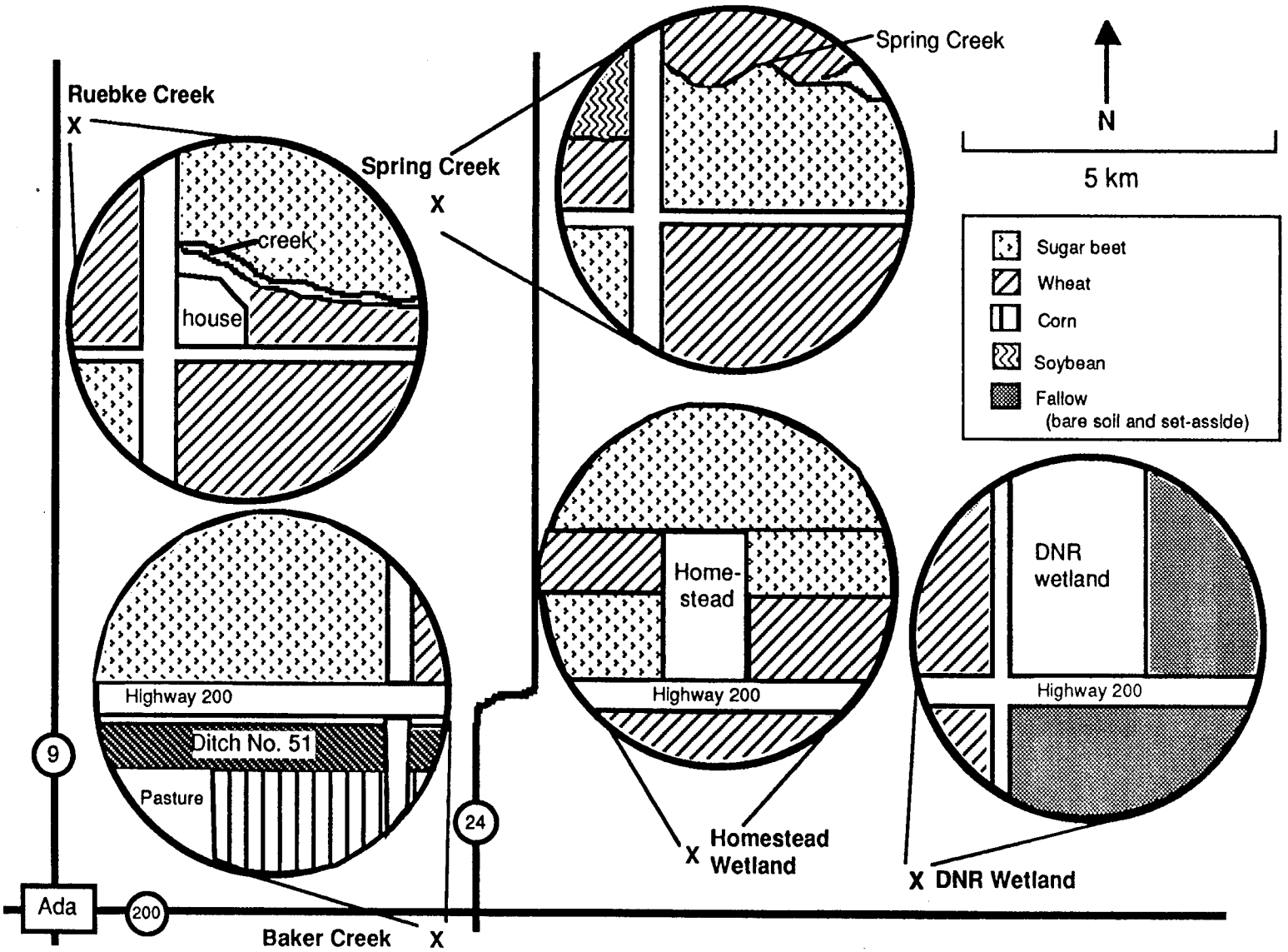

Figure 3. Location of study sites and pattern of land use where nestling birds were sampled, in Norman County, Minnesota in June 1992.

Baker Creek

This site was located in Section 13 of McDonaldsville Township, including part of Judicial Ditch Number 51 (Fig. 3). The ditch was a shallow, channelized waterway flowing westward, parallel to State Highway 200. A cornfield and cow pasture bordered the creek to the south and Highway 200 bordered it to the north. A sugar beet field of approximately 112 ha was directly north of the highway and there were approximately 1.6 ha of non-agricultural habitat at this site. Red-winged blackbird nests at this site were constructed in cattail *(Typha sp.)* in the creek bed, or in willow *(Salix sp.)* or other woody plants on the banks of the waterway. Samples were also collected from house sparrows in a nest under a road bridge at this site. We monitored 17 nests along this creek, which were located approximately 15 to 30 m from the edge of the beet field. The field north of Baker Creek was sprayed from a plane with Lorsban® on 9 June 1992.

Homestead Wetland

The Homestead Wetland was on an abandoned home site in the southwestern quarter of Section 9 in Lake Ida Township (Fig. 3). There was a small wetland (diameter approximately 15 m) on this site and cattail and stinging nettle (*Urtica dioica*) were dominant plant species. There was little water early in the season, but after several days of rain the water level reached approximately 1 m. This wetland was at the center of a plot of non-agricultural land of approximately 16 ha, and was primarily dominated by grasses and low shrubs, although several hectares were dominated by hardwood trees. Three sides of the site were bordered by beet fields and nests were approximately 150 to 250 m from the borders of these fields. Most (7 of 11) red-winged blackbird nests at the site were constructed in cattail, although we found 3 nests in small shrubs (about 1 m high) in the upland surrounding the wetland. The field to the north was band sprayed with Lorsban® on 6 June 1992 and fields to the west of the Homestead wetland were broadcast sprayed on 8 June 1992 with Lorsban®.

Spring Creek

The Spring Creek site was in the southwest quarter of Section 7 in Green Meadow Township (Fig. 3). Nests at this site were along a creek (approximately 2 m wide) with containment dikes built on either side of the waterway. At this site willow and other shrubs covered approximately 50% of the banks; the other 50% were dominated by sedges and grasses. In addition, there were 2 small cattail wetlands (diameter 6 to 10 m) within 10 m of the creek. Non-agricultural habitat at this site covered approximately 16 ha, but nests were located in a 4 ha area adjacent to a sugar beet field (> 40 ha). A wheat field was located across the road to the east and north and 2 more beet fields were south and west of the monitored nests, within 1 km of the site. The 11 nests we monitored here were approximately 50 to 100 m from the nearest beet field. The field to the south of Spring Creek was band sprayed with Lorsban® on 3 June 1992 and 12 June 1992. Large fields to the southwest and south of the site were treated aerially with Lorsban® on 10 June 1992.

Ruebke Creek

Ruebke Creek was located in the southwest corner of Section 10 in Pleasant View Township (Fig. 3) and comprised approximately 1 ha of land dominated by sedges and grasses, with a shallow creek winding through it. There was up to 1 m of standing or slowly moving water early in the season, and about 2 m after several days of rain. North and adjacent to the creek was a sugar beet field (within 100 m of the monitored nests) and to the southwest across a paved road was another large beet field (≈ 80 ha); the other adjacent fields were planted in wheat. A house with a small lawn (≈ 0.2 ha) was located directly south of the creek. Five nests were monitored at this site and were constructed in cattails or small shrubs (< 3 m tall). The sugar beet field north of Ruebke Creek was not sprayed with Lorsban® during this study.

METHODS

Sample Collection

Nestlings were selected as subjects for this study because their exposure to insecticides is related to events in the vicinity of nests. Monitoring exposure in adults is complicated by migration and by the unpredictability of movements of adults that are not nesting. Red-winged blackbirds were the focal species for this study, but house sparrows and brown-headed cowbirds also were sampled to increase plasma sample size. We located nests by searching wetland vegetation and observing adult red-winged blackbird behavior. To assist in monitoring nests we marked vegetation near nests with plastic flagging or placed flagged wires approximately 1.5 m from nests. Nests were observed at intervals of 2-5 days and the number of eggs and chicks were recorded at each visit. Individual nestlings were marked for identification with black magic marker on feathers on the back or wings.

Nestlings were weighed with a Pesola® scale and the length of the tarsus and bill were measured prior to sampling. Blood was drawn from the jugular vein of nestlings with disposable 1 ml syringes and 27 gauge needles. The skin and feathers on the neck near the jugular vein were swabbed with alcohol prior to drawing blood. Needles, syringes, and micro-centrifuge tubes were rinsed with sodium heparin immediately prior to use, and blood was stored on wet ice until centrifuged. Blood was centrifuged within 1 hour of collection for 10 minutes at 3200 RPM in a portable centrifuge powered by a car battery. Carcasses and plasma were stored in a freezer (at approximately -10 C for up to five days) until shipped on dry ice for analysis. Eleven birds were euthanized, after blood was drawn, for ChE analysis of brain tissue and residue analysis of the gastrointestinal tract and carcass washes. The entire gastrointestinal tract, rather than the gut contents or the crop, was analyzed for Lorsban® residue to maximize the opportunity for identifying residue, if present. We sampled 3 birds from Spring Creek a second time 5 days after the first samples were drawn; in all other cases birds fledged or disappeared before a second blood sample was obtained.

Age Estimates

Hatching date was determined by visiting nests at 2 - 5 day intervals and by observations of indications of recent hatching (damp down on nestlings, only one of several eggs hatched when those eggs later hatched, egg shell presence in the nest, etc.). Young observed with signs of recent hatching were considered to have hatched on the day they were first observed. Hatching dates of siblings could subsequently be determined because red-winged blackbird eggs within a clutch hatch within 24 hours of one another (Payne 1969). Mass, wing chord length, and culmen length of all nestlings were measured and recorded at each nest visit, and used to develop regression equations to estimate age of nestling when hatching date was not apparent. Blood samples were collected from red-winged blackbirds that were from 5 to 11 days old because nestlings < 5 days old were too small for drawing blood, and most had fledged by day 11 or 12. Red-winged blackbirds generally fledge between 10 and 14 days after hatching (Payne 1969, Harrison 1984).

Wing chord length has been shown to increase linearly with age during most of the nestling period for several bird species including house martins (*Delichon urbica*), northern harriers (*Circus cyaneus*) (Bryant 1975, Saunders and Hansen 1989) and red-tailed hawks (Petersen and Thompson 1977, Bechard et al. 1985). We feel that we were able to estimate nestling age of red-winged blackbirds accurately to within 1 day based on wing chord measurement. Nestlings estimated to be from 4-6 days old were treated as 5-day-old nestlings in analyses. Similarly, nestlings estimated at 9-11 days old were included in analyses as 10-days old.

Insecticide Application

Landowners and persons farming the property adjacent to study sites were contacted for information regarding insecticide application. Foliar insecticide application at all sites where nestling blood was collected is summarized in Table 1. No additional independent monitoring of insecticide application was attempted. The 1992 season was an outbreak situation for sugar beet

root maggot; approximately 80% of the sugar beet fields in the Ada area were sprayed with Lorsban® during the 1992 growing season, in contrast to most years when only about 3% of fields receive a foliar application of an insecticide (D. Berglund, pers. commun.).

Table 1. Application of Lorsban® insecticide on study sites in Norman County, Minnesota in June 1992. Application was determined by direct observation and through landowner and farmer contact.

Site	Number of Application	Application Method	Distance from Application (m)
Wetland	0	-	>300
Ruebke Creek	0	-	>300
Homestead	2	band and broadcast	150-250
Spring Creek	3	band and aerial	50-100
Baker Creek	1	aerial	15-30

In addition to foliar application of insecticides, sugar beets in northwestern Minnesota receive application of ChE-inhibiting insecticides at planting. Virtually all sugar beet fields in the study area were treated with granular application of Counter® (Turbofos, an OP insecticide) or Lorsban® at planting. Other crops in the area generally do not receive insecticide application at planting (D. Berglund, pers. comm.). In 1992 most sugar beet fields near Ada were planted during or near the first week in May. During late May and June 1992 foliar application of ChE-inhibiting insecticides on crops other than sugar beets was unlikely, however up to 5% of wheat fields may have received OP or carbamate application during our sampling period (D. Berglund, pers. comm.).

Chemical Analyses

Chemical and biochemical analyses were performed at The Institute of Wildlife and Environmental Toxicology, Clemson University. For a detailed description of these methods see Hoff (1992) and Gard and Hooper (1993).

Tissue Preparation :- Samples were stored for 3 months at -80. C prior to chemical analysis. Plasma samples were thawed and diluted 40-fold prior to analysis. Brain tissue was removed from carcasses, mixed with 0.05M tris buffer (pH 7.4) and homogenized for 30 seconds in a VirTis homogenizer. The homogenate was diluted again to 300 fold in the same buffer. Brain and plasma preparations were separated into 3-500 µl aliquots. One aliquot was placed on ice, for approximately 35 minutes, until it was assayed for absolute ChE activities. The other 2 were used to test for ChE reactivation in the presence of 2-PAM. One of the aliquots was spiked with 2-PAM and the other with distilled water, and these samples were assayed after a 30 minutes incubation period. Mean activities from the values of samples run in triplicate were compared with an upper tailed t-test to determine if there was a significant increase in ChE activity in the sample incubated with 2-PAM compared to the sample incubated without 2-PAM. Those samples found to have a significant ($P \leq 0.05$) increase of ChE activity of > 5% after 2-PAM incubation were considered to contain OP-inhibited ChE and therefore had been exposed to an OP.

Cholinesterase Activity Assay :- Diluted brain and plasma samples were spectrophotometrically assayed on a Vmax 96-well Kinetic microplate reader (Molecular Devices Corporation; use of trade name does not imply endorsement by the U.S. Fish and Wildlife Service, or the University of Minnesota) using a modification (Gard and Hooper 1993) of the method of Ellman et al. (1961). All samples were run in triplicate at room temperature (22 - 25 C). The substrate, acetylthiocholine (AThCh, Sigma Chemical Co., St. Louis, MO), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, Sigma), Tris 8.0 buffer, and enzyme dilution were added to microplate wells. Cholinesterases hydrolyze AThCh to thiocholine and acetate and thiocholine attacks DTNB

releasing 5-thio-2-nitrobenzoic acid, a yellow colored chromophore absorbing at 405nm (Hill and Fleming 1982). The rate at which this yellow color is generated directly reflects thiocholine liberation and therefore represents ChE activity, which is expressed as µmoles AThCh hydrolyzed/min ("units")/ml plasma, or /g brain tissue (Ellman et al. 1961). Total plasma ChE was separated into AChE and BChE activities using the selective BChE inhibitor, iso-OMPA (tetraisopropylpyrophosphoramide, Sigma; Aldridge 1953, Fairbrother et al. 1991). Activity remaining after a 5-min incubation with 10^{-4} iso-OMPA is from AChE, and BChE activity is calculated as the difference between measured TChE and AChE activity. Brain ChE is almost entirely AChE (e.g. Hill 1989, Walker and Thompson 1991), thus BChE activity is not reported for brain tissue.

Residue Extraction :- Residue extractions were performed on gastrointestinal-tract (GI-tract) tissue with contents and on solvent used to wash carcasses. GI-tract tissues were dispersed using a VirTis homogenizer and then samples were extracted with a 3:1 hexane:acetone solvent mixture on an orbital shaker. The volume of solvent was reduced by nitrogen evapo-ration. Samples greater than approximately 5 g were divided into samples of ≤ 5 g each and homogenized, extracted, and analyzed separately. A 3:1 solution of hexane:acetone was also used to wash carcasses, which were subsequently separated through gas chromatography. Carcasses were washed by placing them in a sealed glass container with 50 ml of hexane:acetone (3:1) and shaking the container for 2 minutes to ensure the carcass surface was completely saturated. Resulting solvents were passed through dried sodium sulfate in a glass funnel lined with 41 Whatman filter paper and nitrogen evaporation was used to reduce volume prior to gas chromatographic analysis.

STATISTICAL ANALYSIS

Mean activity values among sample sites for each age group (5 and 10-day old nestlings) were compared using 1-way analysis of variance for differences in TChE (total cholinesterase), AChE , and BChE activity and significantly different means were distinguished using a Tukey test. We used Kruskal-Wallis tests to compare the magnitude of reactivation among collection sites. Data from 5 and 10-day old nestlings were evaluated using diagnostic thresholds (DT's) as described by Hill (1988). Samples that exhibited activities 2 standard deviations below the mean of birds collected at the reference site were considered to have been exposed to an OP. Reactivation for reference sites and each treatment and age group was also noted. Reactivation was considered to be significant when a sample incubated with 2-PAM had an increase of > 5% compared to the paired sample without 2-PAM and when the means of the three replicates of each sample were significantly different ($P < 0.05$). Parametric statistical procedures follow those outlined in Snedeor and Cochroan (1980) and nonparametric procedures follow Gibbons (1985).

RESULTS

Sample Collection

Fifty-three blood samples were collected from 50 nestlings (5 house sparrows, 4 brown-headed cowbirds, and 41 red-winged blackbirds). In addition, 10 red-winged blackbirds and 1 house sparrow from which blood had been collected were euthanized for measurement of brain ChE activity and pesticide residue analyses of gut contents and carcass surfaces. Sample collection is summarized in Table 2.

Table 2. Summary of the plasma and carcasses collected from nestling passerine birds in Norman County, Minnesota in June 1992. All plasma samples and brain tissue from all carcasses were analyzed for 2-PAM ChE reactivation and all carcasses were tested for Lorsban® residue. Samples are from red-winged blackbirds unless otherwised noted.

Site	Number of nests sampled	Number of plasma samples	Number of carcasses	Ages (range in days)	Dates of sampling	Dates of Lorsban® application	Number of plasma reactivations	Number of carcasses with Lorsban® residue	Number of samples with brain reactivation
DNR Wetland	6	10	1	5 - 10	9 June 11 June	-	2	0	0
Ruebke Creek[1]	1	2	0	5	20 June	-	0	0	0
Homestead Wetland	4	9	2	4 - 10	19 June 20 June	6 June 8 June	4	0	0
Spring Creek	4	12	2	5 - 10	13 June 18 June 20 June	3 June 10 June 12 June	2	2	0
Baker Creek[2]	5	20	6	5 - 12	10 June 12 June 18 June 20 June	9 June	8	0	1

[1] These values are for brown-headed cowbird nestlings

[2] Four plasma samples and one carcass are from house sparrow nestlings

Age Estimates

Simple linear regression (n = 23) of nestling age against mass, wing chord, and culmen length for red-winged blackbirds of known hatching date indicated that wing chord (Fig. 4) was the best predictor of age, based on R^2, the proportion of variance explained by the simple linear model, and by the pattern of regression residuals. Addition of mass or culmen length did not significantly improve the regression model. We thus estimated age of unknown-age nestling red-winged blackbirds from wing chord measurements [AGE = 2.38 + WING * (0.14), where AGE is measured in days since hatching and WING is wing chord measurement in mm, (R^2 = 0.945, P = 0.000)].

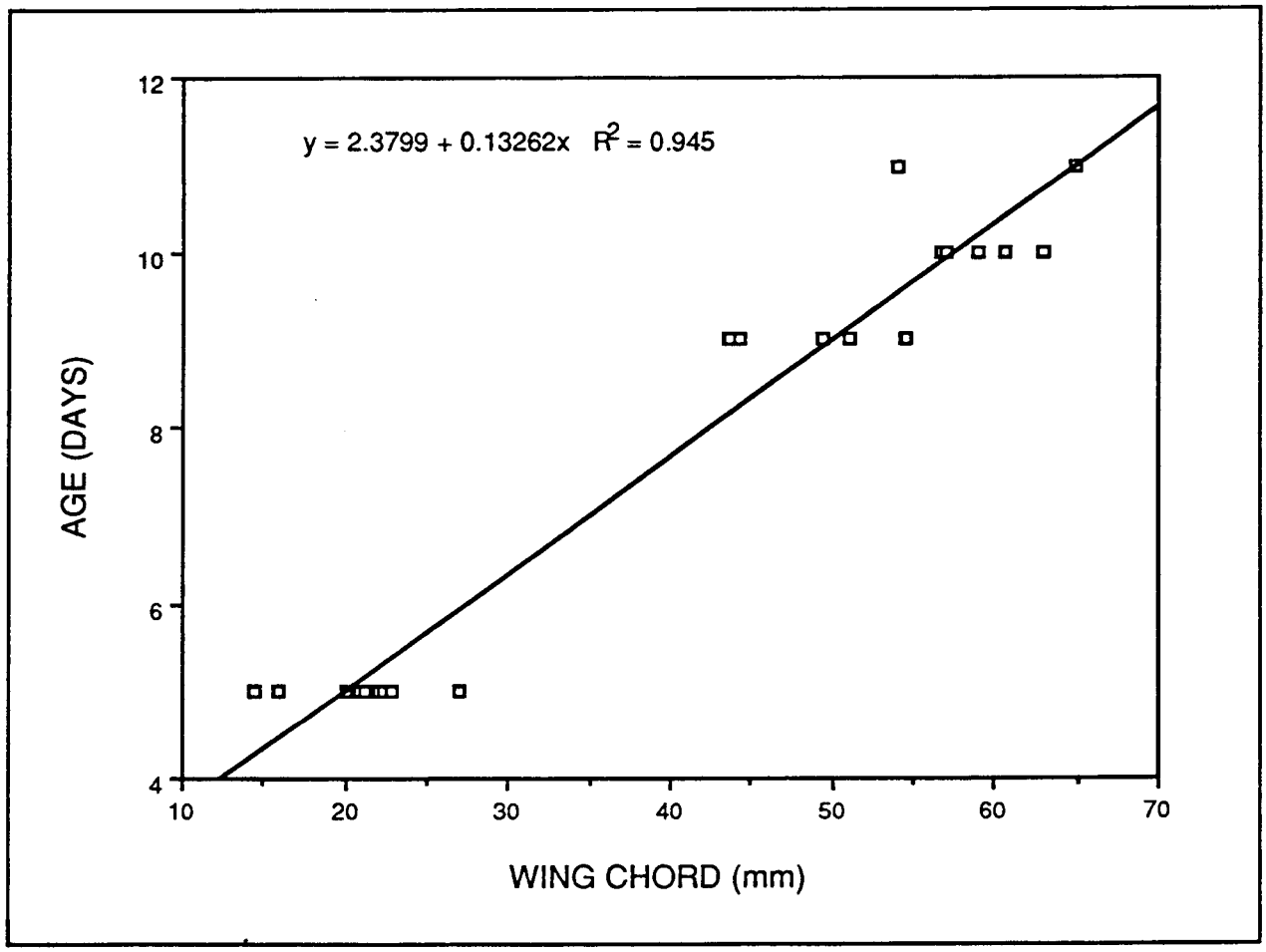

Figure 4. Simple linear regression of age versus wing chord for red-winged blackbird nestlings (n = 23) of known age, sampled in Norman County, Minnesota, June 1992.

Plasma Analyses

Absolute Cholinesterase Activity Levels:- Blood samples from the Spring Creek site had significantly lower TChE (1-way ANOVA, $F_{3,19}$ = 12.80, $P < 0.001$) and BChE ($F_{3,19}$ = 10.35, $P < 0.001$) activities than samples from the DNR Wetland and Homestead sites for red-winged blackbird nestlings that were approximately 5 days old. AChE activity levels ($F_{3,19}$ = 3.15, $P = 0.049$) from 5-day old red-winged blackbird nestlings on the Spring Creek site were lower than those from the Homestead site. Total ChE (t = -2.51, *d.f.* = 21, $P = 0.033$) and BChE (t = -2.56, *d.f.* = 21, $P = 0.043$) activity were lowest in 5-day-old nestlings at sites adjacent to treated beet fields. There were no differences among sites in TChE, AChE, or BChE activity for 10-day-old nestlings (all P's > 0.375, n = 19). In general, TChE activity levels increased with age for nestlings at most sites ($r = 0.421$, $P = 0.004$, Fig. 5). Acetylcholinesterase activity (r =-0.324, $P = 0.032$, Fig. 6) tended to decrease with age and BChE activity (r = 0.482, P = 0.001, Fig. 7) increased with age. We were able to resample red-winged blackbirds from one nest during this study. We drew blood from these birds at 5 days and again at 10 days old. AChE, BChE, and TChE activity for these birds at both sampling dates are shown in Figure 8.

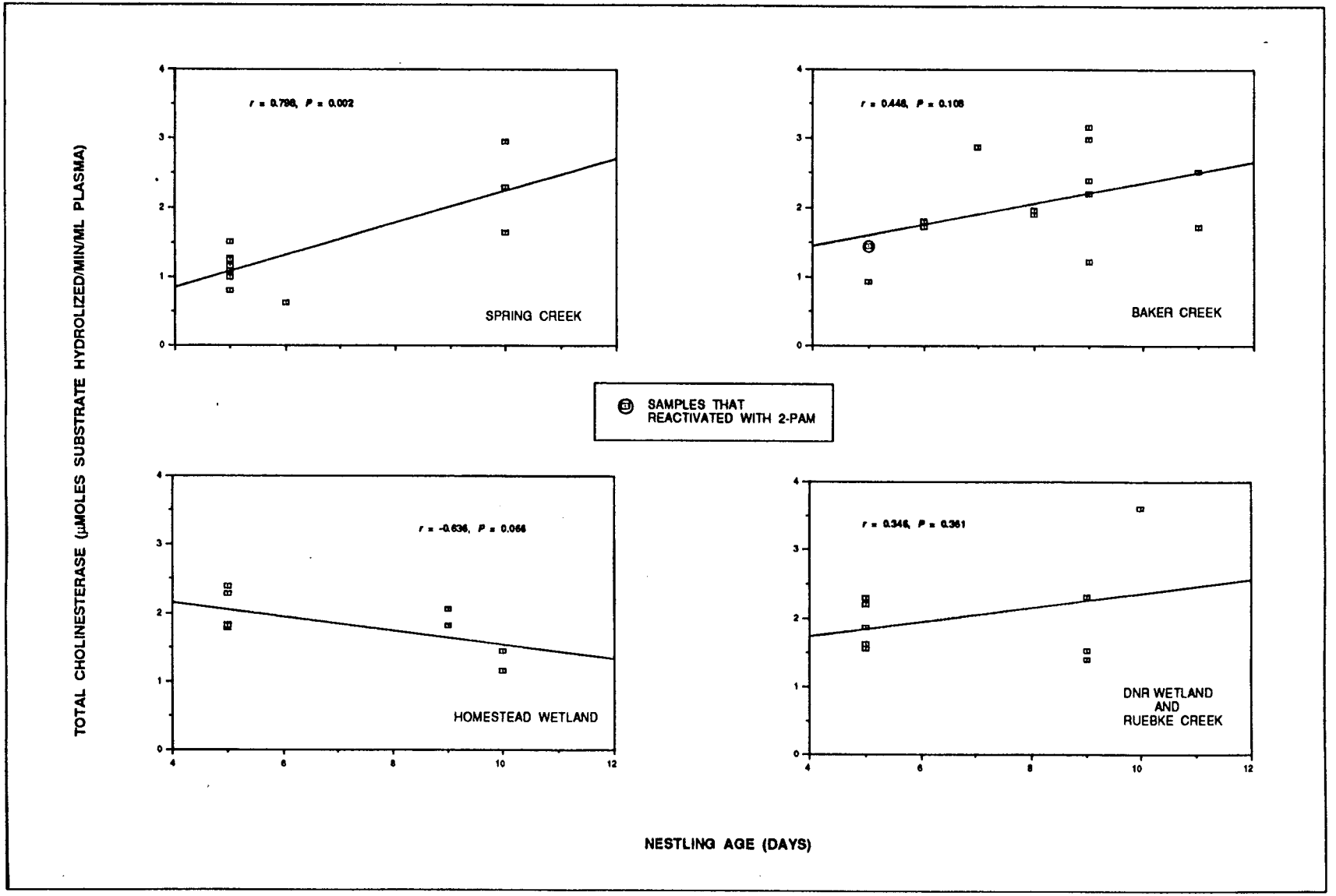

Figure 5. Total cholinesterase activity levels in plasma of nestling red-winged black as a function of age for samples collected in Norman County, Minnesota in June 1992. Days post-insecticide application vary for birds that are the same age.

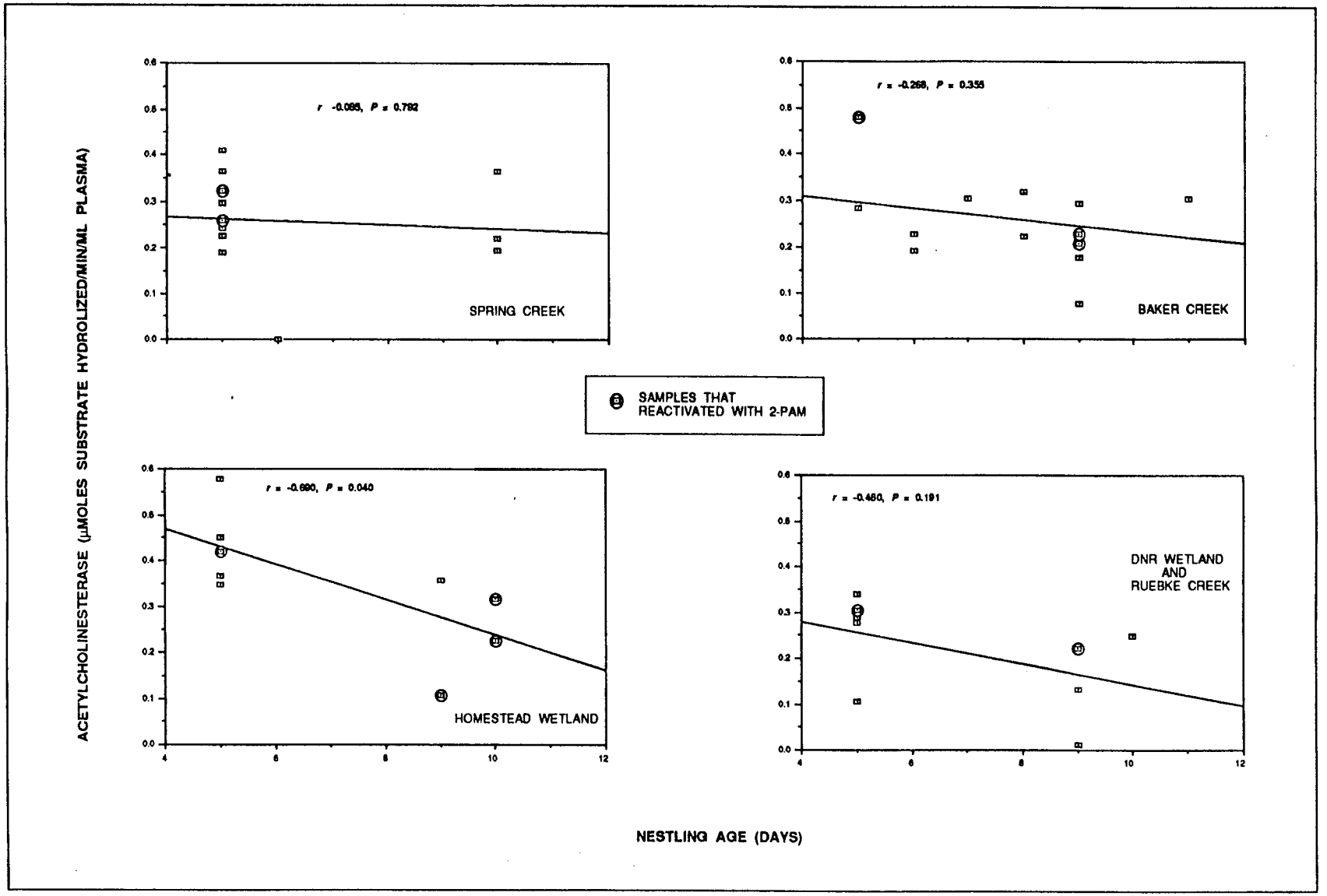

Figure 6. Acetylcholinesterase activity levels in plasma of nestling red-winged blackbirds as a function of age for samples collected in Norman County, Minnesota in June 1992. Days post-insecticide application vary for birds that are the same age.

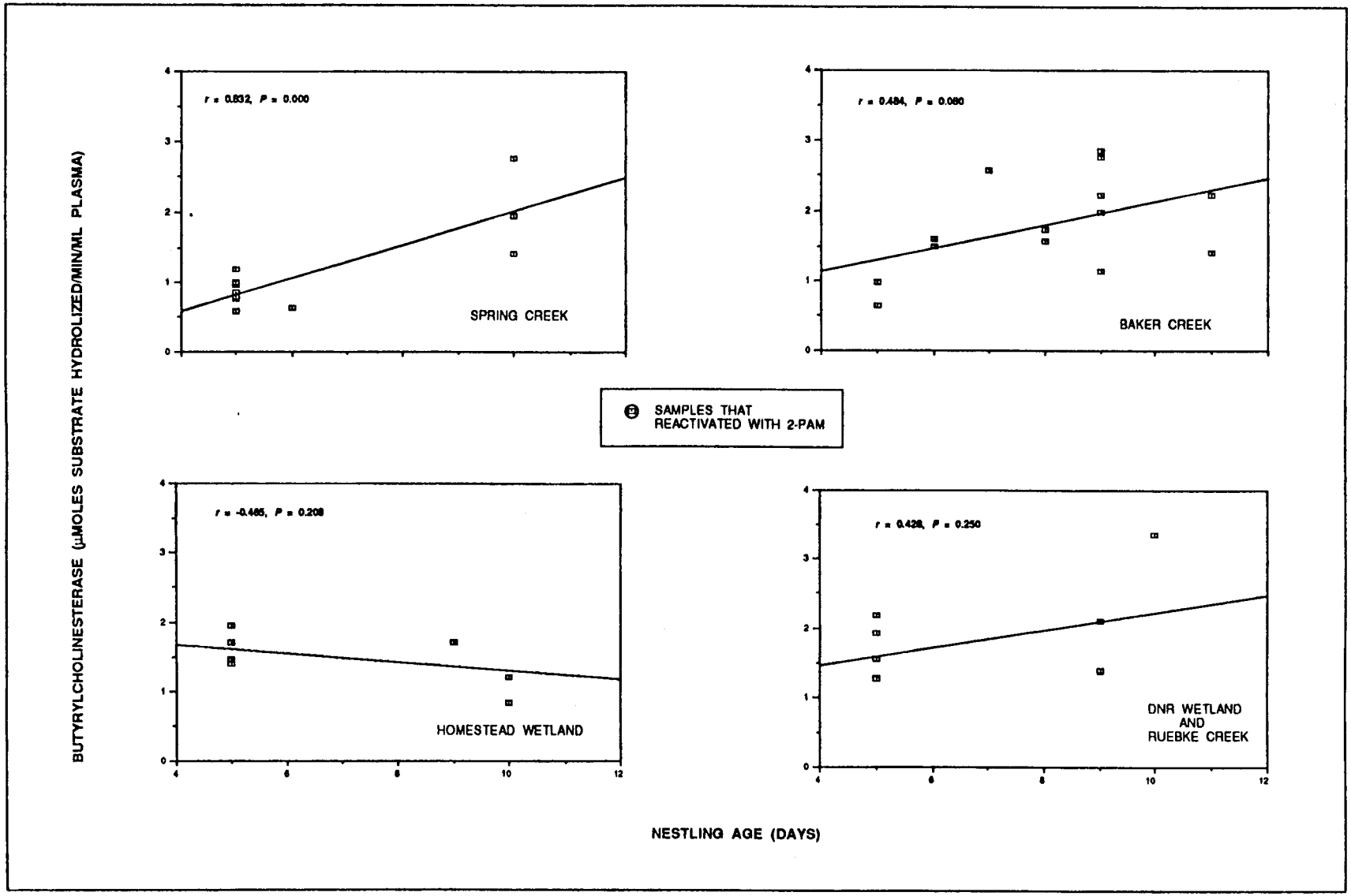

Figure 7. Butyrylcholinesterase activity levels in plasma of nestling red-winged blackbirds as a function of age for samples collected in Norman County, Minnesota in June 1992. Days post-insecticide application vary for birds that are the same age.

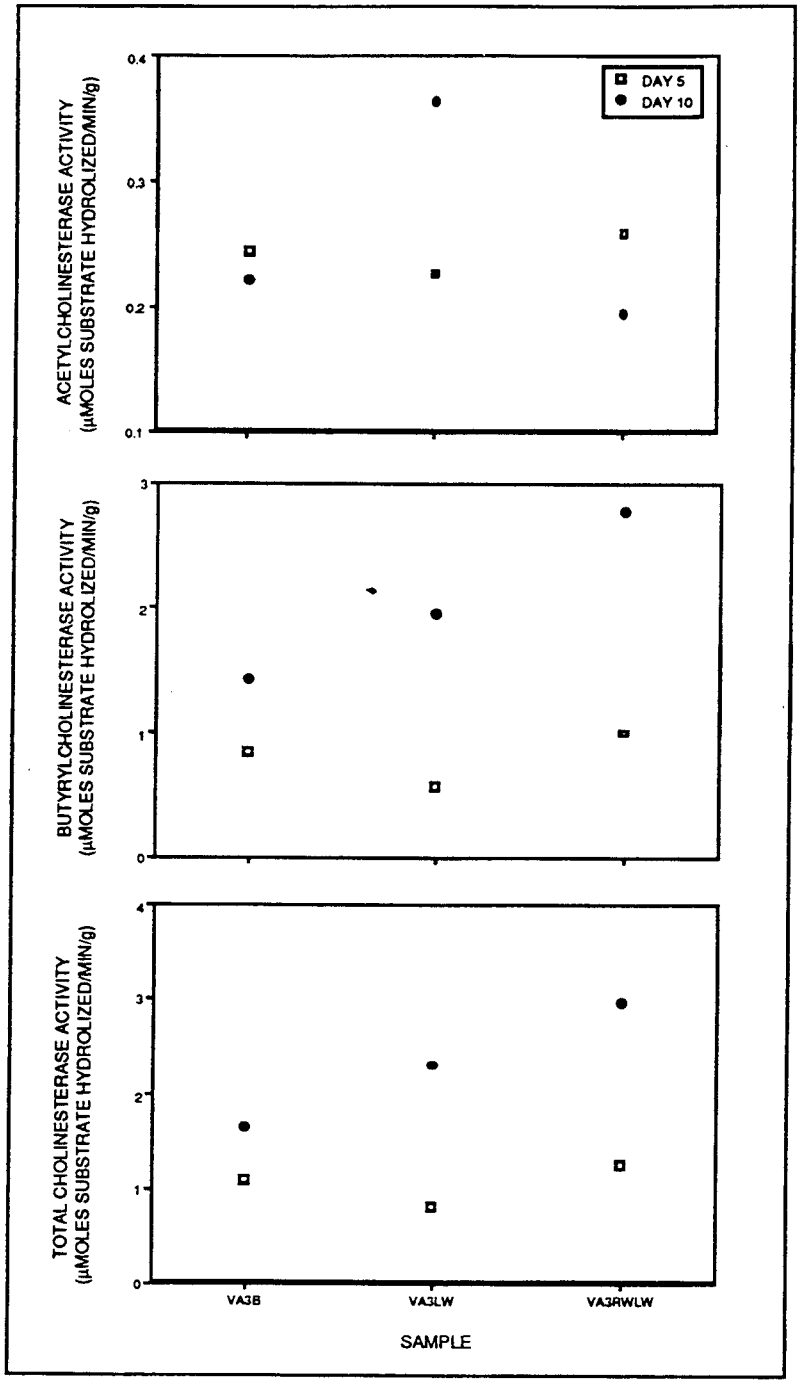

Figure 8. Plasma acetylcholinesterase, butyrylcholinesterase, and total cholinesterase activities for 3 red-winged blackbird nestlings that were sampled twice in June 1992, in Norman County, Minnesota. The birds were from 1 nest at the Spring Creek site, and were sampled on the same days, at approximately 5 and 10 days after hatching. Sample VA3RWLW reactivated 14% with the addition of 2-PAM on day 5.

Diagnostic Thresholds:- We calculated diagnostic thresholds (2 standard deviations below the mean) for TChE, AChE, and BChE activity for treatment sites (Figs. 9-11) for plasma samples drawn from red-winged blackbirds that were approximately 5 days old. At the reference sites (DNR Wetland and Ruebke Creek), 2 plasma AChE sample reactivated in the presence of 2-PAM, indicating exposure to an OP. Diagnostic thresholds were calculated with and without samples that reactivated with the addition of 2-PAM. Mean TChE, AChE, and BChE activity for the samples collected from 5-day-old nestlings at the reference sites were 1.82, 0.30, and 1.52 µmoles substrate hydrolyzed/min/ml plasma respectively, and without samples that reactivated the means were 1.69, 0.34, and 1.35 µmoles substrate hydrolyzed/min/ml plasma respectively. At the Baker Creek site TChE activity in 1 sample was below both calculated diagnostic thresholds (Fig. 9). At the Spring Creek site 3 samples had TChE activity below the threshold for all reference samples and 2 were below the threshold calculated excluding the reactivating samples (Fig. 10). One sample from Spring Creek had an AChE activity below both thresholds and BChE activity from 1 sample was below the threshold for all samples, but not for the threshold calculated excluding reactivating samples. No samples were below the calculated diagnostic threshold at the Homestead site (Fig. 11). In total, 4 samples had TChE activities and 1 sample had BChE activity below the threshold calculated from all reference samples and AChE activity in 1 sample was below both calculated thresholds.

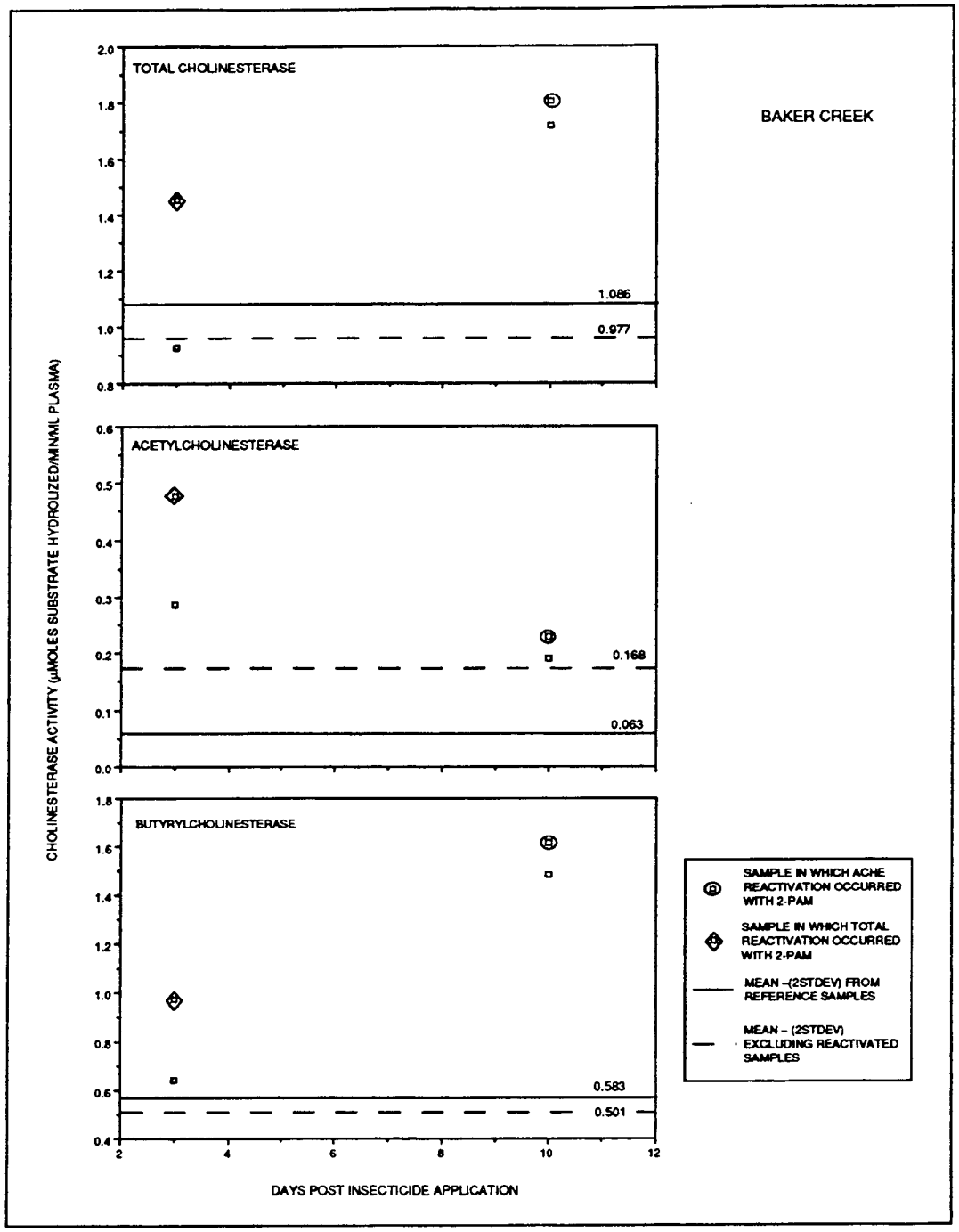

Figure 9. Cholinesterase activity levels and diagnostic thresholds with (solid line) and without (dashed line) reactivating samples for plasma samples from 5-day-old red-winged blackbirds collected at the Baker Creek study site in Norman County, Minnesota in June 1992. Diagnostic thresholds were calculated from samples from 5-day-old nestlings collected at the DNR Wetland site.

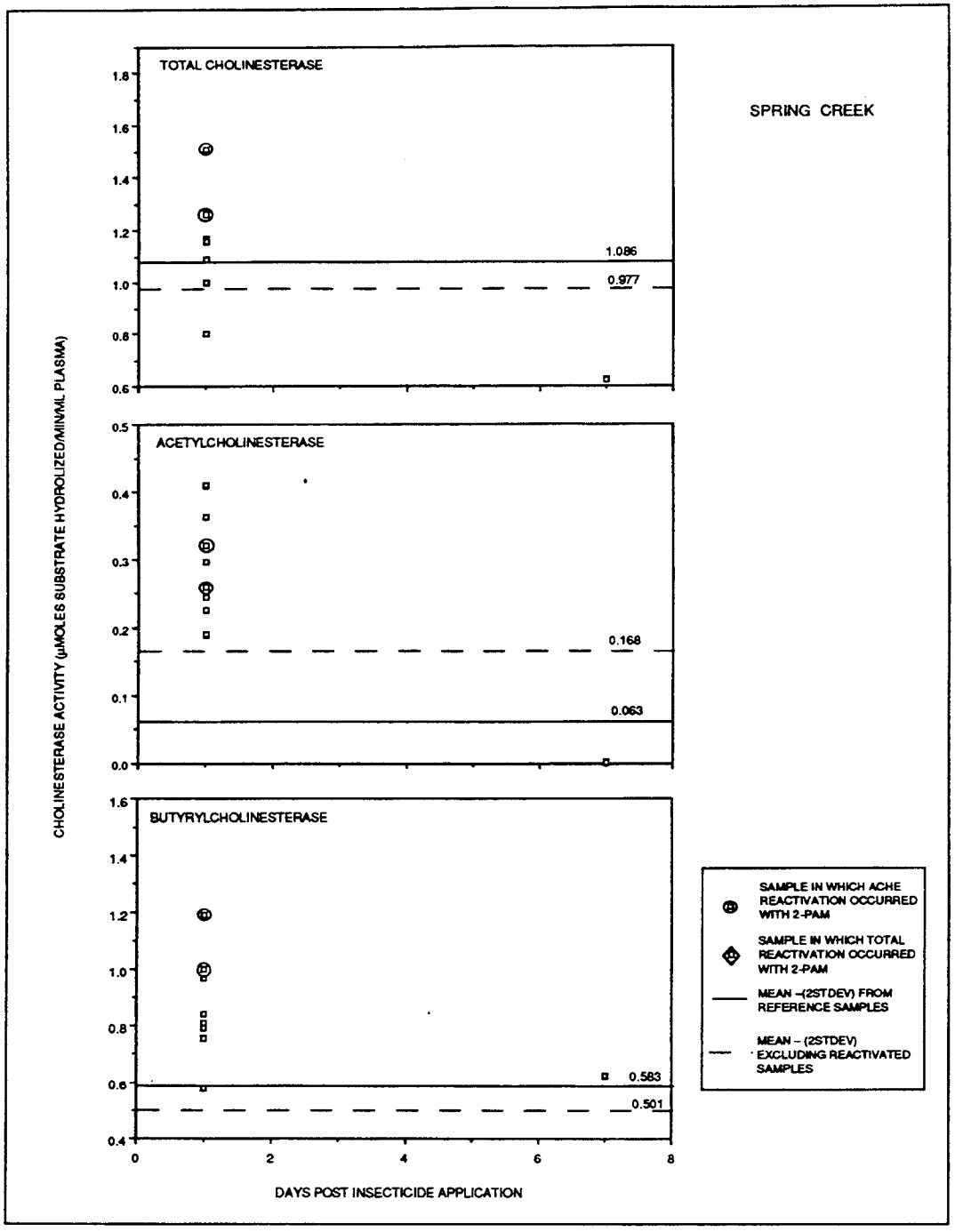

Figure 10. Cholinesterase activity levels and diagnostic thresholds with (solid line) and without (dashed line) reactivating samples for plasma samples from 5-day-old red-winged blackbirds collected at the Spring Creek study site in Norman County, Minnesota in June 1992. Diagnostic thresholds were calculated from samples from 5-day-old nestlings collected at the DNR Wetland site.

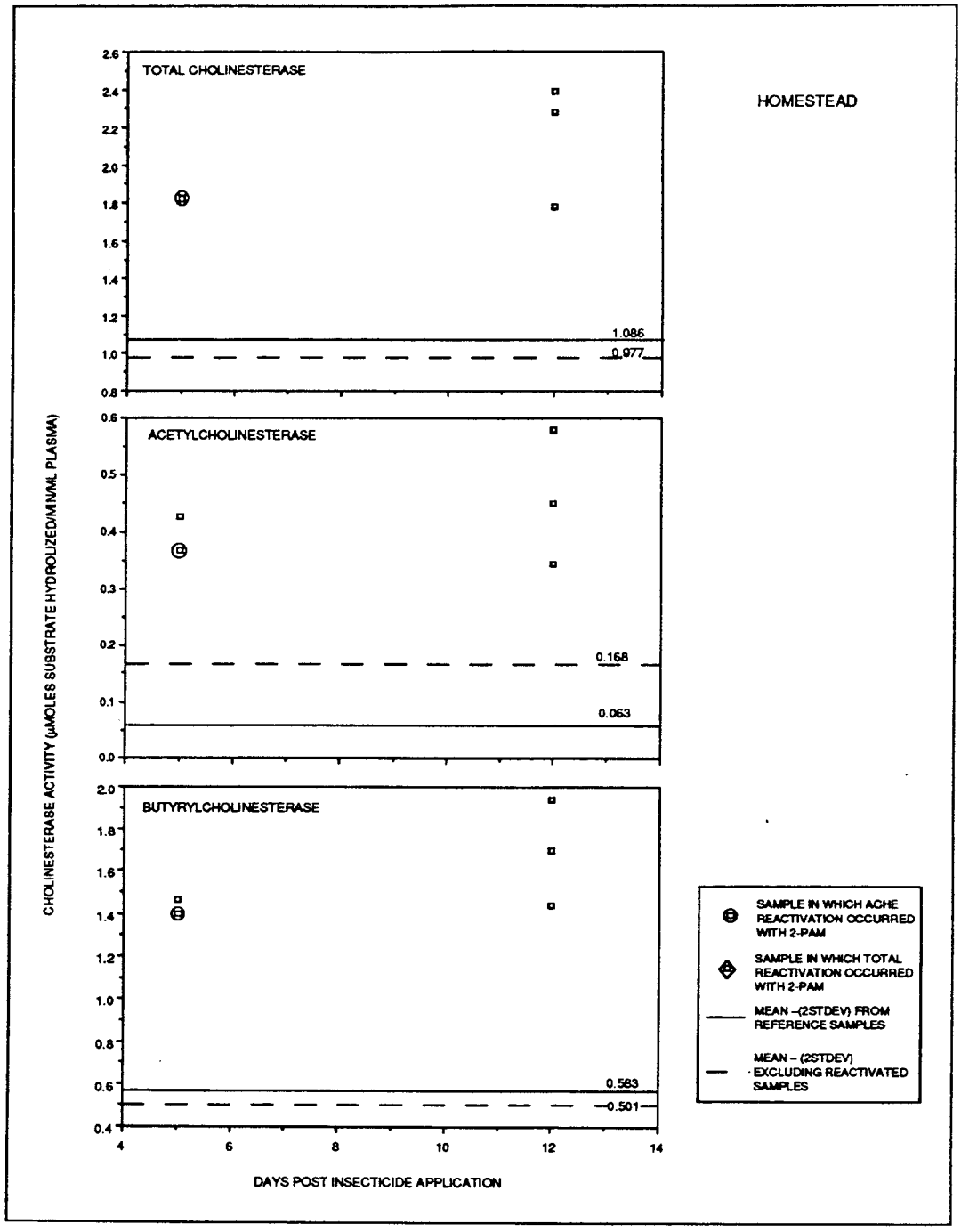

Figure 11. Cholinesterase activity levels and diagnostic thresholds with (solid line) and without (dashed line) reactivating samples for plasma samples from 5-day-old red-winged blackbirds collected at the Homestead study site in Norman County, Minnesota in June 1992. Diagnostic thresholds were calculated from samples from 5-day-old nestlings collected at the DNR Wetland site.

2-PAM Reactivations:- Sixteen (31%) of the 52 plasma samples exhibited a statistically significant ($P < 0.05$) increase of ≥ 5% in AChE activity after the addition of 2-PAM (Fig. 12). Seventeen percent of the samples from the DNR Wetland and Ruebke Creek sites combined (distant from Lorsban® application) reactivated. Seventeen percent of the samples from the Spring Creek site reactivated, 42% of the samples from Baker Creek reactivated, and 44% from the Homestead site reactivated. Nestlings from sites that were near fields where Lorsban® was applied were more likely to exhibit plasma AChE reactivation than nestlings from reference sites where OP or carbamate insecticide application was improbable ($X^2 = 3.805$, $d.f. = 1$, $P \approx 0.05$). TChE reactivated in 30% of samples from Baker Creek, and in all cases where TChE reactivated, AChE also reactivated ≥ 5%. The magnitude of AChE reactivation at Baker Creek also appeared higher than at other sites (Kruskal-Wallis $H = 7.65$, $d.f. = 3$, $P = 0.054$). Nest-specific reactivation occurred on both the Baker Creek and Homestead sites, with individuals from the same nest exhibiting AChE reactivation ≥ 5%. The highest increases in AChE reactivation occurred within 1-3 days after insecticide application (Fig. 13), although there was no significant relationship between days post application and magnitude of reactivation ($r = -0.298$, $P = 0.301$).

Plasma samples that exhibited significant reactivation after incubation with 2-PAM did not appear to fall outside of the range of ChE activity levels for samples from other similar-aged birds (Figs. 5-7). In plasma from individuals that were sampled twice, AChE from one bird (VA3RWLW, Fig. 8) at 5 days old reactivated 14% with the addition of 2-PAM, but plasma from the same bird showed no reactivation 5 days later. No samples that exhibited reactivation with 2-PAM were also below calculated diagnostic thresholds (Figs. 9-11).

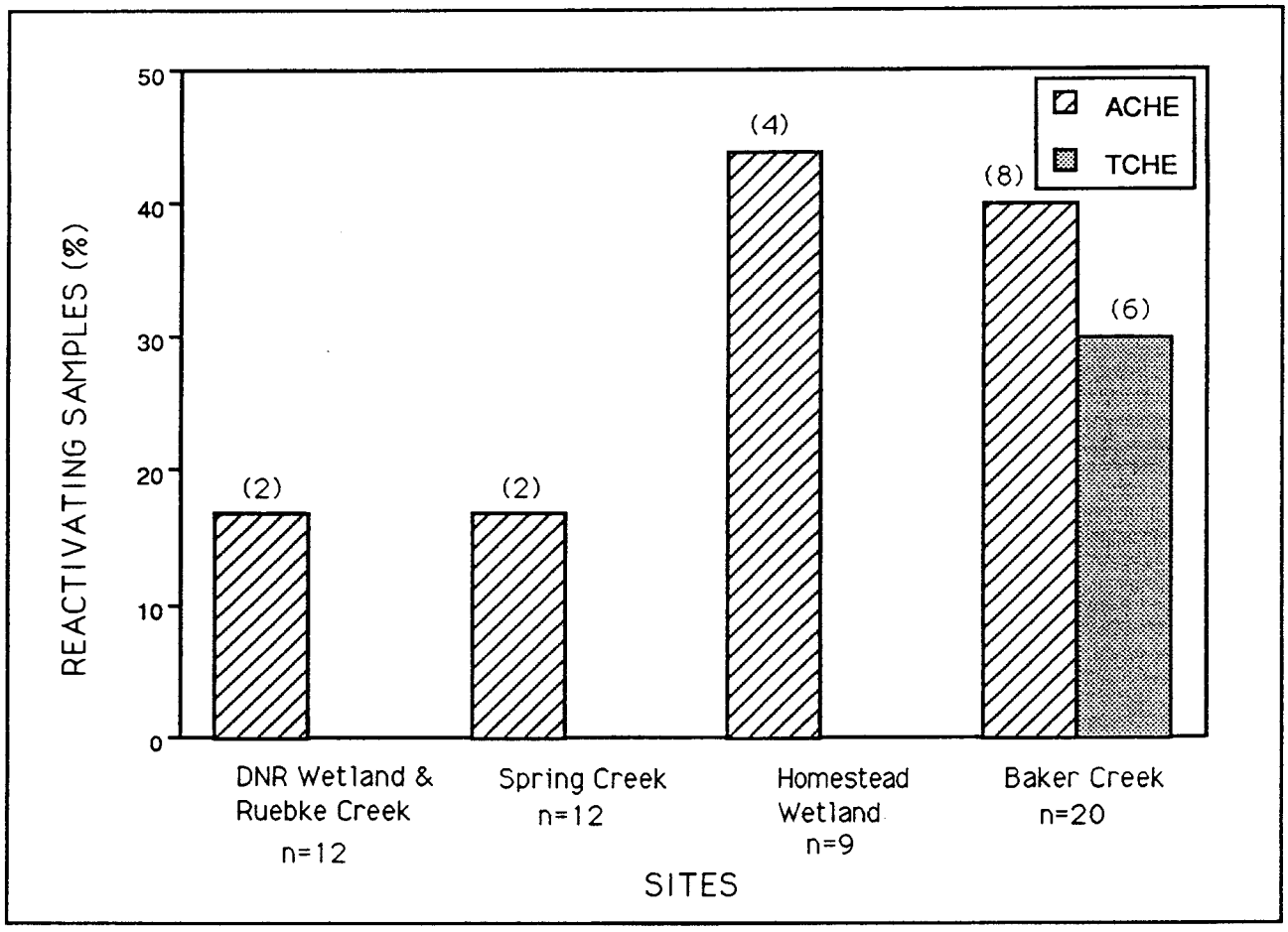

Figure 12. Percent of nestling plasma samples in which significant reactivation of acetylcholinesterase (AChE) and total cholinesterase (TChE) was observed. The total number of birds sampled at each site (n) is noted below site names and the number of samples that reactivated are above the bars. Red-winged blackbird, house sparrow, and brown-headed cowbird nestlings were sampled in Norman County, Minnesota in June 1992 (see Table 2).

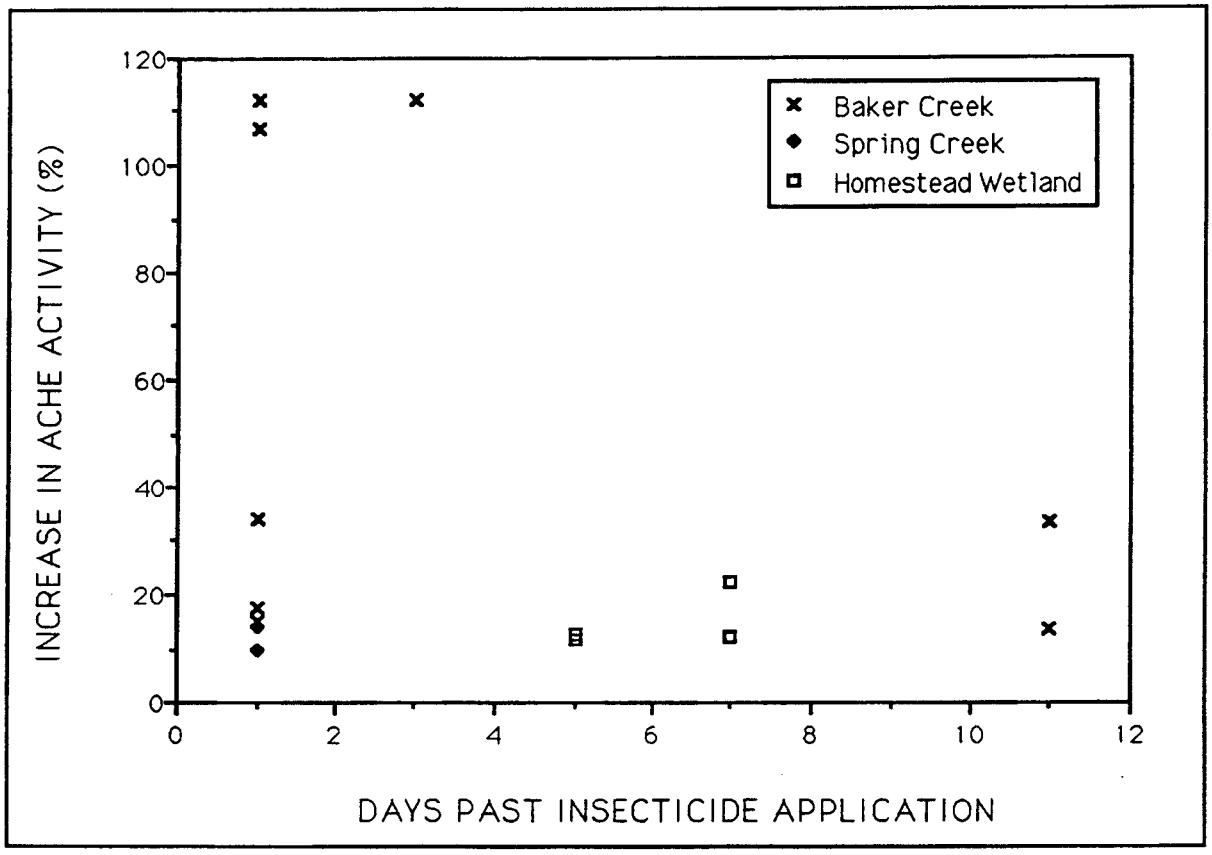

Figure 13. Percent increase in acetylcholinesterase (AChE) activity where significant (≥ 5%) reactivation occurred in plasma samples of nestling red-winged blackbirds as a function of days past Lorsban® application. Nestlings were sampled in Norman County, Minnesota in June 1992.

Brain Tissue Analyses

Brain tissue from 10 nestling red-winged blackbirds and 1 house sparrow (Table 3) was analyzed for TChE activity and reactivation with the addition of 2-PAM. Total ChE reactivated significantly in 1 brain from a red-winged blackbird nestling from the Baker Creek site; plasma TChE and AChE also reactivated from this individual. In addition, 2 plasma samples from individuals where brain tissue had been sampled exhibited significant reactivation of both AChE and TChE, and 2 additional plasma samples exhibited significant reactivation of AChE (Table 3). Total ChE activity (primarily AChE) in brain tissue increased significantly with age (Fig. 14).

Table 3. Plasma TChE and AChE reactivation and brain AChE reactivation for all nestlings in Norman County, Minnesota in June 1992.

Species[1]	Site	Age	Reactivation			Residue detected in or on carcass	Date sprayed with Lorsban®	Date Sampled
			TChE (plasma)	AChE (plasma)	Brain TChE			
RWBB	DNR Wetland	5	*[2]	*	-	-	-	13 June
HOSP	Baker Creek	unknown	Y[3]	Y	-	Y	9 June	10 June
RWBB	Baker Creek	11	-	-	-	-	9 June	18 June
RWBB	Baker Creek	5	-	-	-	-	9 June	12 June
RWBB	Baker Creek	6	-	-	-	-	9 June	20 June
RWBB	Baker Creek	5	Y	Y	Y	Y	9 June	12 June
RWBB	Baker Creek	8	-	-	-	-	9 June	20 June
RWBB	Spring Creek	5	-	-	-	-	3,10,12 June	13 June
RWBB	Spring Creek	5	-	Y	-	-	3,10,12 June	13 June
RWBB	Homestead Wetland	5	-	-	-	-	6 & 8 June	20 June
RWBB	Homestead Wetland	10	-	Y	-	-	6 & 8 June	20 June

[1] RWBB = Red-winged blackbird, HOSP= house sparrow

[2] '*' indicates no plasma sample was drawn

[3] 'Y' indicates reactivation occurred or residue was detected in the sample.

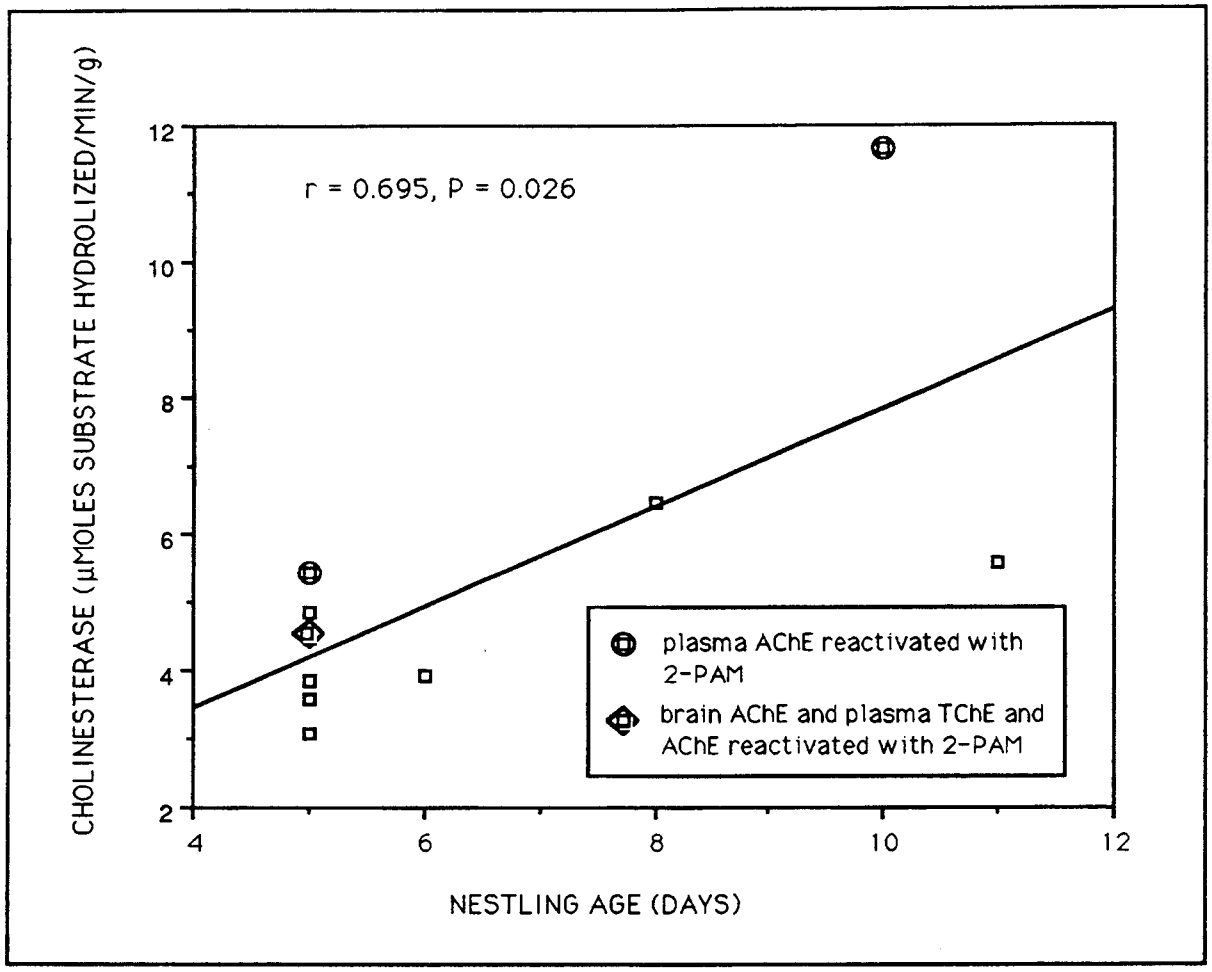

Figure 14. Brain AChE activity as a function of nestling age for red-winged blackbirds (n=10) collected in Norman County, Minnesota in June 1992.

Residue Analyses

Lorsban® residues were identified from 1 carcass wash and 2 GI-tracts in birds collected at Baker Creek. Lorsban® was recovered from the carcass wash (0.135 µg) and GI-tract material (0.538 µg) in one house sparrow . Trace levels (< 0.1 µg) of Lorsban® were identified in the GI-tract sample of 1 red-winged blackbird, which also exhibited 2-PAM reactivation of TChE in brain tissue.

DISCUSSION

Insecticides

Farmers we contacted were generally responsive to our requests for information related to Lorsban® application on fields adjacent to the study sites. In addition we observed warning notices posted around fields where Lorsban® was applied aerially. Quantitative estimates of the rates of insecticides reaching crops and soils in fields and greater certainty regarding compounds reaching fields of interest can be obtained by analysis of compounds collected on filter paper cards placed in the fields prior to insecticide application, although in this study we were primarily interested in whether insecticides had been applied and not application rate. Given that OP insecticide application occurred on most (> 80 %) of sugar beet fields in the study area in 1992, we felt confident that nests near beet fields were near pesticide application.

Plasma Analyses

In 1992 we were able to sample nestling birds in an agricultural landscape in northwestern Minnesota, and evaluate the feasibility of monitoring exposure to ChE-inhibiting insecticides through measuring blood plasma and brain ChE activity. Total ChE and BChE activity levels were lowest for 5-day-old red-winged blackbirds at the Spring Creek site compared to other sites. Combining samples from sites where insecticide application occurred, TChE and BChE activity levels were lower at treated sites compared to reference sites. AChE levels did not differ between samples collected at treated versus reference sites. These data suggest that TChE and BChE activity levels may indicate exposure to ChE-inhibiting insecticides when treated and non-treated sites are compared.

BChE is a general cholinesterase that can catalyze reactions with a variety of substrates and AChE is a specific enzyme that catalyzes acetylcholine hydrolysis. In birds, the proportion of plasma ChE these different enzymes comprise varies for different species (Hooper and Wilson 1987) and species have different age-dependent patterns of AChE and BChE activity. The

significance of these patterns in relation to ChE-inhibition is not well understood. Plasma AChE declined slightly and BChE increased over the nestling period in bluebirds (*Sialia sialis*) and European starlings (*Sturnus vulgaris*) (Gard and Hooper 1993). In similar work, Grue et al. (1981) observed an increase in European starling ChE activity over the nestling period. We observed a similar pattern of AChE and BChE activity in red-winged blackbirds. Although sample size was small our results are consistent with other research, which suggests that our sampling and laboratory procedures were comparable to those of previous studies. Nestling starlings also have been shown to be more sensitive to exposure to OP's than adults (Grue and Shipley 1984). Gard and Hooper (1993) suggested that the increase in plasma BChE activity during the nestling period may be responsible for decreased sensitivity to ChE-inhibitors that occurs in some birds during the same period (Grue and Shipley 1984). Our analyses is further complicated by the fact that many of the sampled birds had been exposed to OP's at different times and at different ages.

The diagnostic threshold method of measuring exposure to ChE-inhibiting insecticides did not appear to be a sensitive indicator of exposure in this study. The activity of 6 ChE samples fell > 2 standard deviations below the mean of reference samples, and none of these exhibited significant reactivation in the presence of 2-PAM. Five samples were within 2 standard deviations of the mean ChE activity in reference samples yet exhibited significant reactivation in the presence of 2-PAM. However, this analysis was hindered by relatively small size of samples of similar-aged birds.

Reactivation of ChE in plasma samples appeared to be a sensitive indicator of insecticide exposure; nestlings from sites that were near fields where Lorsban® was applied were more likely to exhibit ChE reactivation than nestlings from reference sites where OP or carbamate insecticide application was improbable (Fig. 13). The magnitude of ChE reactivation was highest within 1-3 days of insecticide application, although reactivation was measured up to 11 days after application of Lorsban® (Fig. 13). In general, AChE reactivation appeared to be a more sensitive indicator of exposure to ChE-inhibiting insecticides than TChE reactivation. In all

cases where TChE reactivated, AChE also reactivated, although the reverse was not true. One particularly important characteristic of the 2-PAM reactivation technique is the comparison of a single sample's ChE activity with and without the addition of 2-PAM. Thus, results are independent of factors, such as age and species differences, that influence the initial level of enzyme activity (Hooper 1988), and remove the requirement of a non-exposed reference sample.

It is unclear why 2 samples from the reference sites distant from ChE-inhibiting insecticide application showed plasma reactivation. Perhaps these birds were exposed to an application of which we were unaware. It is impossible to rule out the possibility that nestlings at all sites, including the reference site, were exposed to ChE-inhibiting insecticides other than the foliarly applied Lorsban®, or pesticide drift from other fields in the area; drift from aerial pesticide application has been shown to occur over distances at least 1 to 2.5 km (Akesson and Yates 1964, Deely 1980), and aerially applied 2,4-D has reportedly drifted up to 24 km (Akesson and Yates 1964). Studies of chlorpyrifos have estimated the half-life in soils to be from 8 to 279 days, averaging 69 days (Marshall and Roberts 1978). However, in another study of 2-PAM reactivation of plasma from birds (Hooper 1988), unexposed birds showed low rates and levels of reactivation. Low levels of reactivation may not necessarily signal exposure to ChE-inhibiting compounds, and additional laboratory investigation to estimate the frequency of false positives is indicated.

Brain Tissue Analyses

Only 1 of 11 (9%) brain samples collected exhibited significant ChE reactivation (Table 3). Three (27%) plasma samples collected from birds that were euthanized exhibited significant TChE reactivation, and 5 (46%) plasma samples exhibited significant AChE reactivation. Both TChE and AChE reactivated from plasma collected from the nestling where brain ChE reactivated, and AChE reactivated in all 3 cases where TChE reactivated (Table 3). These results suggest that plasma AChE reactivation may be a more sensitive indicator of ChE-

inhibiting insecticide exposure than either plasma TChE reactivation or brain ChE reactivation. Studies indicate that plasma ChE inhibition generally occurs at lower levels of OP exposure than does brain ChE inhibition (Ludke et al. 1975, Fleming 1981, Fleming and Grue 1981, Hill and Fleming 1982); this may explain why more plasma than brain samples reactivated.

Residue Analyses

Residue analyses of GI-tracts and wash samples indicated exposure to Lorsban® in 2 of 11 (18%) carcasses submitted for analysis; 1 red-winged blackbird and 1 house sparrow. In the red-winged blackbird, plasma TChE and AChE and brain ChE exhibited significant reactivation. The house sparrow plasma TChE and AChE exhibited significant reactivation, however brain ChE in this sample did not reactivate (Table 3).

The occurrence of Lorsban® in GI-tracts and wash samples suggests that nestlings were exposed to insecticides by adults that foraged where prey had been exposed, or that adults themselves were exposed directly and transferred the insecticide to nestlings. The single nestling that had detectable levels of Lorsban® residues in both its GI-tract and in a wash sample was from a nest located beneath a bridge at the Baker Creek site, where direct overspray or drift were highly unlikely. Additionally, we observed the female red-winged blackbird from the nest where brain ChE reactivated in a nestling, foraging in a beet field that had been treated with Lorsban®. Both of these observations suggest that adults may forage in areas where exposure to insecticides is likely, and that in turn, nestlings can be exposed to insecticides through ingestion of prey delivered by adults and through direct contact with parents. Several studies (Grue and Shipley 1984, Wolfe 1992) have shown that in some altricial species nestlings are significantly more sensitive to OP's than adults. Thus, it is possible that adults could continue to forage in fields with contaminated insects and remain behaviorally unaffected , while delivering doses to their nestlings that cause ChE inhibition.

Vulnerable Bird Species

To fully assess the magnitude of the risk to birds of exposure to agricultural OP's and carbamates, it is necessary to determine which species of birds occur in agricultural landscapes. In Minnesota most agricultural landscapes were originally prairie, brushland, parkland or hardwood forest habitats, and birds that nest and forage in the vicinity of agricultural lands are predominantly grassland, brushland, and parkland species. A list of these species is presented in Appendix I.

In Minnesota over 99% of the original prairie habitat has been converted to agricultural or other uses (Coffin and Pfannmuller 1988) and populations of many bird species native to prairie habitats have declined. In addition, more birds (n=20) native to prairie habitats are on the state list of endangered, threatened or special concern species than for any other habitat type in the state (Pfannmuller and Coffin 1989). Baird's sparrow (*Ammodramus bairdii*), burrowing owl (*Athene cunicularia*), chestnut collared longspur (*Calcarius ornatus*), and Sprague's pipit (*Anthus spragueii*) were classified as endangered; the loggerhead shrike (*Lanius ludovicianus*) was listed as threatened and the following were listed as special concern: American bittern (*Botaurus lentiginosus*), American white pelican (*Pelecanus erythrorhynchos*), common moorhen (*Gallinula chloropus*), Forster's tern (*Sterna forsteri*), greater prairie-chicken (*Tympanuchus cupido*), Henslow's sparrow (*Ammodramus henslowii*), horned grebe (*Podiceps auritus*), king rail (*Rallus elegans*), marbled godwit (*Limosa fedoa*), sandhill crane (*Grus canadensis*), sharp-tailed sparrow (*Ammodramus caudacutus*), short-eared owl (*Asio flammeus*), upland sandpiper (*Bartramia longicauda*), Wilson's phalarope (*Phalaropus tricolor*), and the yellow rail (*Coturnicops noveboracensis*). None of Minnesota's prairie birds are included on the Federal list of endangered or threatened species.

CONCLUSIONS

1. Benefits and drawbacks of methods as field monitoring tools

We used several different laboratory and statistical procedures for evaluating exposure of nestling birds to the foliar application of Lorsban® in sugar beet fields. These analytical methods have benefits and drawbacks related to use as monitoring tools (Table 4). The 2-PAM reactivation technique of plasma seemed to most frequently identify nestlings that may have been exposed to Lorsban®. The benefits of comparing a sample ChE activity before and after the addition of 2-PAM rather than collecting reference samples is important because it is often difficult to identify with certainty birds that have not been exposed to ChE-inhibiting chemicals. It also reduces the number of birds that may need to be sampled. The most significant drawback is that this technique has not commonly been applied to this type of problem and the frequency of false positives has not been established. For example, in this study two of the reference samples reactivated and it is unclear whether those birds were actually exposed to ChE-inhibitors.

2. Birds are exposed

Our results suggest that red-winged blackbird and house sparrow nestlings were exposed to a ChE-inhibiting compound, probably Lorsban®, that was applied to sugar beet foliage. The route of exposure may have been via the adult, either through direct contact or from food items delivered to the young. This study was not designed to address the consequences of exposure. However, some studies suggest that behavioral and physiological effects of sublethal exposure may impact survival (Martin et al. 1991*b*, Patnode and White 1991, Galindo et al. 1985, Rattner and Franson 1984).

3. Potential topics for further work

There is a need for additional research related to monitoring exposure of birds to ChE-inhibiting compounds and the consequences of exposure for these organisms. For the purpose of monitoring, it is important to continue to develop the technique of 2-PAM reactivation. It is necessary to understand the occurrence of reactivation in reference samples and to standardize the methods and interpretations of these results. In addition, we believe that the use of nest boxes to attract nesting birds could be a convenient method of collecting samples from wild birds in specific habitats.

A full understanding of the importance of the exposure of birds to these insecticides cannot be determined without further evaluation of the effects of exposure of birds to these compounds. Additional laboratory and field studies should address whether exposure to OP and carbamate insecticides significantly impacts behavior, reproductive success or survival in birds.

Table 4. Comparison of methods of evaluating exposure to cholinesterase-inhibiting insecticides in birds.

METHODS OF EVALUATING EXPOSURE	Benefits	Drawbacks
ABSOLUTE CHE ACTIVITY	• established methodology with examples present in the literature • measures inhibition of all ChE-inhibiting insecticides	• large sample sizes required • unexposed birds are required for comparison • age of nestlings must be determined • ChE activity may be low because of factors other than insecticide exposure
CHE REACTIVATION	• each individual serves as its own control • smaller sample sizes may be adequate • relative amount of reactivation may be compared • if ChE activity is low for reasons other than exposure to inhibitors reactivation may not occur	• false positives may occur • some insecticides are not reversible, do not show reactivation • there are few data available for comparison • effective for a short period of time after exposure (this could also be a benefit - for identifying chemicals responsible for exposure)
THRESHOLD COMPARISONS	• identification of outliers	• insensitive measure of exposure • large sample size is required

Continued...

Table 4. Continued from previous page.

MEASUREMENT OF CHOLINESTERASE ACTIVITY

Benefits	Drawbacks
RESIDUE ANALYSIS	
• positive link between specific compound and exposure	• probably short term indicator of exposure
• indicator of routes of exposure	• generally destructive sampling
	• qualitative indication of exposure
PLASMA	
• non-destructive sampling	• relationship of response to toxicity is unknown
• repeatable	• short term response in blood
• quick response to exposure	• plasma ChE is more variable than brain tissue ChE
• exposure occurs after relatively small doses	
BRAIN	
• long term response	• response usually occurs only after larger doses
• response is believed related to toxicology of contaminants	• destructive sampling
• brain ChE is less variable than plasma ChE	• longer time between exposure and inhibition

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APPENDIX I

Bird species found in or near prairie habitats and agricultural fields in Minnesota, Iowa or Illinois. Breeding status in Minnesota is presented in the column titled "Breeding", 'R' indicates the birds breed in Minnesota, 'M' indicates they are present in Minnesota prairie areas as migrants.

Species	Sources	Breeding[f]
Horned grebe (*Podiceps auritus*)	e	R
American white pelican (*Pelecanus erythrorhynchos*)	e	R
Northern harrier (*Circus cylaneus*)	d	R
Red-tailed hawk (*Buteo jamaicensis*)	a,c,d	R
Swainson's hawk (*Buteo*)	d	R
American kestrel (*Falco sparvarius*)	d	R
Northern bobwhite (*Colinus virginianus*)	a,c	R
Sharp-tailed grouse (*Tympanuchus phasianellus*)	f	R
Greater prairie-chicken (*Tympanuchus cupidio*)	d	R
Ring-necked pheasant (*Phasianus colchius*)	a,c,d	R
Gray partridge (*Perdix perdix*)	a,c,d	R
American bittern (*Botaurus lentiginosus*)	d	R
Least bittern (*Ixobrychus exllis*)	d	R
Great blue heron (*Ardea herodias*)	d	R
Black-crowned night-heron (*Nycticorax nycticorax*)	d	R
Green-backed heron (*Butorides striatus*)	c,d	R
Great egret (*Casmerodius albus*)	d	R
Killdeer (*Charadrius vociferus*)	a,b,c,d	R
Semipalmated plover (*Charadrius semipalmatus*)	d	M
Lesser golden-plover (*Pluvialis dominica*)	c,d	M
Upland sandpiper (*Bartramia longicauda*)	a,d,e	R
Pectoral sandpiper (*Calidris melanotos*)	d	M
Least sandpiper (*Calidris minutilla*)	d	M
Spotted sandpiper (*Actitis macularia*)	d	R
White-rumped sandpiper (*Calidris fuscicollis*)	d	M
Baird's sandpiper (*Calidris bairdii*)	d	M
Semipalmated sandpiper (*Calidris pusilla*)	d	M
Sanderling (*Calidris alba*)	d	M
Dunlin (*Calidris alpina*)	d	M
Short-billed dowitcher (*limnodromus griseus*)	d	M
Greater yellowlegs (*Triga melanoleuca*)	d	M
Lesser yellowlegs (*Tringa flavipes*)	d	M
Hudsonian godwit (*Limosa haemastica*)	d	M
Marbled godwit (*Limosa fedoa*)	d,e	R
Common moorhen (*Gallinula chloropus*)	e	R
Virginia rail (*Rallus limicola*)	d	R
Sora (*Porzana carolina*)	d	R
Yellow rail (*Coturnicops noveboracensis*)	d,e	R
King rail (*Rallus elegans*)	e	R

APPENDIX I (cont.) Bird species found in or near prairie habitats and agricultural fields in Minnesota, Iowa or Illinois.

Species	Sources	Breeding[f]
American coot (*Fulica americana*)	d	R
Common snipe (*Gallingo gallinago*)	d	R
Wilson's phalarope (*Phalaropus tricolor*)	d,e	R
American woodcock (*Scolopax minor*)	d	R
Canada goose (*Branta canadensis*)	d	R
Blue-winged teal (*Anas discors*)	d	R
Green-winged teal (*Anas crecca*)	d	R
American wigeon (*Anas americana*)	d	R
Gadwall (*Anas strepera*)	d	R
Northern Pintail (*Anas acuta*)	d	R
Northern shoveler (*Anas clypeata*)	d	R
Mallard (*Anas platyrhnchos*)	d	R
American blackduck (*Anas rubripes*)	d	R
Canvasback (*Athya vallisineria*)	d	R
Redhead (*Athya americana*)	d	R
Ruddy duck (*Oxyura jamaicensis*)	d	R
Ring-necked duck (*Athya collaris*)	d	R
Lesser scaup (*Athya affinis*)	d	R
Ring-billed gull (*Larus delawarensis*)	d	R
Franklin's gull (*Larus pipixan*)	d	R
Forster's tern (*Sterna forsteri*)	e	R
Black tern (*Chlidonias niger*)	d	R
Rock dove (*Columba livia*)	c	R
Mourning dove (*Zenaida macroura*)	a,c,d	R
Black-billed cuckoo (*Coccyzus erythropthalmus*)	d	R
Great horned owl (*Bubo virginianus*)	a,d	R
Short-eared owl (*Asio flammeus*)	d,e	R
Burrowing owl (*Speotyto cunicularia*)	e	R
Northern flicker (*Colaptes auratus*)	c,d	R
Pileated woodpecker (*Dryocopus pileatus*)	c	R
Red-bellied woodpecker (*Melanerpes carolinus*)	c	R
Red-headed woodpecker (*M. erythrocephalus*)	c	R
Hairy woodpecker (*Picoides villosus*)	d	R
Downy woodpecker (*Picoides pubescens*)	c,d	R
Eastern kingbird (*Tyrannus tyrannus*)	a,c,d	R
Western kingbird (*Tyrannus verticalis*)	d	R
Alder flycatcher (*Empidonax alnorum*)	d	R
Least flycatcher (*Empidonax minimus*)	d	R
Great crested flycatcher (*Myiarchus crinitus*)	c	R
Eastern phoebe (*Sayornis phoebe*)	c	R
Willow flycatcher (*Empidonax traillii*)	c,d	R
Eastern wood-pewee (*Contopus sordidulus*)	c	R

APPENDIX I (cont.) Bird species found in or near prairie habitats and agricultural fields in Minnesota, Iowa or Illinois.

Species	Sources	Breeding[f]
Horned lark (*Eremophila alpestris*)	a,b,c,d	R
Tree swallow (*Iridoprocne bicolor*)	a,d	R
Barn swallow (*Hirundo rustica*)	a,b,c,d	R
Cliff swallow (*Petochelidon pyrrhonota*)	a,d	R
Blue jay (*Cyanocitta cristata*)	c,d	R
American crow (*Corvus brachyrhynchos*)	a,c	R
Black-capped chickadee (*Parus atricapillus*)	a,c	R
Tufted titmouse (*P. bicolor*)	c	R
White-breasted nuthatch (*Sitta carolinensis*)	c	R
House wren (*Troglodytes aedon*)	c,d	R
Carolina wren (*Thryothorus ludovicianus*)	c	M
Sedge wren (*Cistothorus platensis*)	a,c,d	R
Marsh wren (*Cistothorus palustris*)	d	R
Gray catbird (*Sumetella carolinensis*)	c,d	R
Brown thrasher (*Toxostoma rufum*)	a,c,d	R
Sprague's pipit (*Anthus spragueii*)	e	R
Loggerhead shrike (*Lanius ludovicianus*)	e	R
American robin (*Taurdus migratorius*)	a,b,c,d	R
Wood thrush (*Hylocichla mustelina*)	a	R
Veery (*Catharus fuscescens*)	d	R
Eastern bluebird (*Sialia sialis*)	c,d	R
Cedar waxwing (*Bombycilla cedrorum*)	c	R
European starling (*Sturnus vulgaris*)	a,cd	R
Red-eyed vireo (*Vireo olivaceus*)	c,d	R
Warbling vireo (*Vireo gilvus*)	d	R
Tenessee warbler (*Dendroica petechia*)	c,d	R
Yellow warbler (*Dendroica petechia*)	c,d	R
Yellow-rumped warbler (*Dendroica coronata*)	d	R
Palm warbler (*Dendroica palmarum*)	d	R
Wilson's warbler (*Wilsonia pusilla*)	d	R
Northern waterthrush (*Selurus noveboracensis*)	d	R
Common yellowthroat (*Geothlypis trichas*)	a,b,c,d	R
Yellow-breasted chat (*Icteria virens*)	c,d	R
House sparrow (*Passer domesticus*)	a,b,c	R
Boblink (*Dolichonyx oryzivorus*)	a,c,c	R
Eastern meadowlark (*Sturnella magna*)	a,c	R
Western meadowlark (*Sturnella neglecta*)	a,b,c,d	R
Red-winged blackbird (*Agelaius phoeniceus*)	a,b,c,d	R
Yellow-headed blackbird (*Xanthocephalus xanthocephalus*)	d	R
Brewer's blackbird (*Euphagus cyanocephalus*)	d	R
Orchard oriole (*Icterus spurius*)	c,d	R
Northern oriole (*I. galbula*)	a,c,d	R
Common grackle (*Quiscalus quiscula*)	a,b,c,d	R

APPENDIX I (cont.) Bird species found in or near prairie habitats and agricultural fields in Minnesota, Iowa or Illinois.

Species	Sources	Breeding[f]
Brown-headed cowbird (*Molothrus ater*)	a,b,c,d	R
Northern cardinal (*Cardinalis cardinalis*)	a,c	R
Rose-breasted grosbeak (*Pheucticus ludovicianus*)	a,c,d	R
Indigo bunting (*Passerina cyanea*)	a,b,c	R
Dickcissel (*Spiza americana*)	a,b,c,d	R
American goldfinch (*Carduelis tristis*)	a,c,d	R
Lapland longspur (*Calcarius lapponicus*)	d	M
Chestnut collared longspur (*Calcarius ornatus*)	e	R
Lark bunting (*Calamospiza melanocorys*)	d	M
Baird's sparrow (*Ammodramus bairdii*)	e	M
Sharp-tailed sparrow (*Ammodramus caudacutus*)	d	R
Savannah sparrow (*Passerculuc sandwichensis*)	a,c,d	R
Grasshopper sparrow (*Ammodramus savannarum*)	a,c,d	R
Vesper sparrow (*Pooecetes gramineus*)	a,b,c,d	R
Henslow's sparrow (*Ammodramus henslowii*)	d	R
Lark sparrow (*Chondestes grammacus*)	c	R
Field sparrow (*Spizella pusilla*)	a,c	R
Song sparrow (*Melospiza melodia*)	a,c,d	R
Chipping sparrow (*Spizella passerina*)	b,c	R
White-crowned sparrow (*Zonotrichia leucophrys*)	c	M
LeConte's sparrow (*Ammodramus leconteii*)	d	R
Clay-colored sparrow (*Spizella pallida*)	d	R
Swamp sparrow (*Melospiza georgiana*)	d	R

a,b Bryan, G.G. and L.B. Best. 1991. Bird abundance and species richness in grassed waterways in Iowa rowcrop fields. Am. Midl. Nat. 126:90-102. (Birds observed in grassed waterways (a) and crop fields (b) in Iowa, 15 May - 31 July 1987 and 1988.)

c Best, L. B., R.C. Whitmore, and G.M. Booth. 1990. Use of cornfields by birds during the breeding season: The importance of edge habitat. Am. Midl. Nat. 123:84-99. (Birds observed in corn fields and in adjacent edge habitats during the breeding season, in Iowa and Illinois, 6 May - 9 July 1986.)

d Jeanne Holler, unpublished data. U.S. Fish and Wildlife Service, Region 3, Refuges and Wildlife. (Birds were identified in prairie remnants in the Rothsay area Minnesota, May to a July 1989.)

e Minnesota Department of Natural Resources. 1986. Checklist of endangered and threatened animal and plant species of Minnesota. Minnesota Natural Heritage Program/ Nongame Wildlife Program, St. Paul, 23 pp.

f Janssen, R.B. 1987. Birds in Minnesota. University of Minnesota Press, Minneapolis. 352-pp.