

THE EFFECTS OF LOW LEVELS OF 2,4-D ON SUNFLOWER  
AND RAGWEED SEED PRODUCTION IN NORTHEASTERN KANSAS

by

DANIEL HOWARD PLETSCHER

B. S., University of Minnesota

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## INTRODUCTION

Farming practices were generally beneficial to game species prior to World War II. Fields and farms were small and interspersed among woodlands. This resulted in a habitat diversity where many game species thrived. Weeds were common in cropland and disturbed areas. Bobwhite (Colinus virginianus) was one species that did well in this type of habitat. Their populations declined after the 1940's (Rosene 1969) when fields became larger and diversity declined. The use of selective herbicides also began shortly after World War II, reducing weed numbers. Many of these plants produce seeds which are utilized by bobwhite.

Two weeds that are very important to the bobwhite in Kansas, common sunflower (Helianthus annuus) and ragweed (Ambrosia artemisiifolia), are very susceptible to 2,4-dichlorophenoxyacetic acid (2,4-D), one of the most-used herbicides in the United States. Ragweed is an especially unpopular weed due to its hayfever-producing pollen. Despite ragweed's value as an important wildlife food (Martin et al. 1951), Sylwester (1951:20) has stated "Any step we can take in the elimination of such unwanted plants as...ragweed should be encouraged."

While weed control is undoubtedly necessary in agricultural land, drift from herbicide spray application can be a problem (Nordby and Skuterud 1974). Drift can affect not only adjacent susceptible crops, but also seed producing weeds in nearby wildlife lands. Low levels of a synthetic auxin herbicide such as 2,4-D may increase or decrease plant growth depending on the level of herbicide used and the conditions under which it was applied. It is the purpose of this study to simulate drift on sunflowers and ragweed, and to determine the subsequent effect on seed production and quality.

The fruits of sunflower and ragweed are technically cypselas, but for simplicity will be called seeds in this report.

The literature on 2,4-D is voluminous. The more pertinent literature on each topic is reviewed in the following section.



## LITERATURE REVIEW

Much of the early work in developing synthetic growth-regulating hormones (including 2,4-D) as herbicides was conducted by the United States Army during World War II. The potential for biological warfare (the destruction of enemy crops) was the basis for this research. The war ended before these herbicides could be so used, but this research facilitated the early post-war introduction of phenoxy herbicides for agricultural purposes (House et al. 1967).

Over 43.5 million pounds (19.7 million kilograms) acid equivalent of 2,4-D were produced in the United States in 1970 (Fowler and Mahan 1975), and in Kansas, 2,4-D is the most-dispersed herbicide by the State Weed and Pesticide Division (Kansas Department of Agriculture 1975:mimeo). This herbicide is used to control broadleaf weeds on range lands and in tolerant crops. The rates used generally vary between 1/2 and 2 pounds acid equivalent per acre (0.56 and 2.24 kg/ha) for barley, corn, oats, pastures, range land, sorghum, and wheat (Klingman et al. 1975:215).

### Mode of Action

Robertson and Kirkwood (1969, 1970) review the mode of action of phenoxy-acid herbicides. After entering the leaf through either the cuticle or stomata, they indicate that the herbicide probably moves through the xylem within the treated leaf. It is then transported throughout the plant via the phloem system. Although 2,4-D affects plant respiration (inhibits oxygen uptake and ATP synthesis), photosynthesis (reduction of CO<sub>2</sub> absorption and inhibition of the Hill reaction), and mineral uptake, the probable primary mechanism of 2,4-D action is the increased synthesis of RNA and protein. This causes the often-seen stem and leaf epinasty and swelling of stems. Holm and Abeles (1968) have suggested that ethylene production in

response to 2,4-D treatment may be the cause of stem swelling and nucleic acid synthesis.

Growth is stimulated by low concentrations of 2,4-D and inhibited by high concentrations. Hanson and Slife (1969) argue that 2,4-D acts as an auxin but accumulates to higher concentrations than indoleacetic acid (IAA) because of its slow degradation. Either growth or inhibition of growth can occur depending on the tissues and concentrations of 2,4-D involved (Wort 1964).

#### Factors Affecting Action of 2,4-D

Humidity, light, and temperature are important in the absorption of herbicides. Pallas (1958) found that significantly more 2,4-D was absorbed and translocated in red kidney beans (Phaseolus vulgaris) at high humidities (70-74 percent) than at low humidities (34-48 percent) within a temperature range of 20-35°C. Clor et al. (1962) found increased translocation at high humidity using cotton (Gossypium barbadense) as a test plant. Water stress in bean plants did not greatly affect absorption of 2,4-D, but translocation was drastically reduced (Basler et al. 1961). They speculated that since stem elongation ceases under moisture stress, endogenous auxins are likely not translocated either. An actively growing plant is necessary for good translocation of an herbicide.

Numerous studies have been conducted concerning the increased activity of 2,4-D with increased temperature. For example, Muzik (1965) showed that tomato (Lycopersicon esculentum) leaves dipped in 2,4-D and kept at 70 and 80°F (21.1 and 26.6°C) exhibited typical 2,4-D responses (epinasty of leaves, twisting and swelling of stems, and tumorous outgrowths). Plants kept at 55°F (12.8°C) showed no visible response to the herbicide; growth continued at the same slow rate as controls. However, symptoms did

show up if the plants were transferred to 70-90°F (21.1-32.2°C) as long as two months after spraying. Pallas (1958) demonstrated an increase in absorption and translocation of 2,4-D with increasing temperatures between 20 and 30°C using red kidney beans. Barrier and Loomis (1957) increased absorption of 2,4-D in soybeans (Glycine max) by increasing temperature. This effect was also found by Marth and Davis (1945) in early winter cress (Barbarea vernea) and narrow-leafed plantain (Plantago lanceolata), and in red kidney beans, perennial rye, and crabgrass (Digitaria sp.) by Kelly (1949).

Light intensity affects the growth rate of plants and therefore is also important in the absorption and translocation of 2,4-D. Generally, plants kept under low light conditions are more susceptible to the herbicide than plants kept under high light conditions. Blackman and Robertson-Cunningham (1955) found that the LD<sub>50</sub> for plants was reduced by a factor of 10 under conditions of 12 percent of normal daylight intensity. Mitchell and Brown (1946) found similar results in bean plants and speculated that the translocation of 2,4-D was closely related to the translocation of organic food materials. Water hyacinth (Eichhornia crassipes) plants treated with 2,4-D also showed greater epinasty and necrosis in shaded plants (Penfound and Minyard 1947), although red kidney beans showed herbicide effects to a lesser extent. Etiolated bean plants did not translocate 2,4-D unless sugar in solution was applied to the leaves (Rohrbaugh and Rice 1949), further substantiating Mitchell and Brown's (1946) results.

The formulation of the herbicide and any surfactants used have an obvious effect on the action of that herbicide. Amine salts and esters are the most common formulations of 2,4-D used (Klingman et al. 1975:216). Although esters are generally more efficiently absorbed by leaves than amines (Robertson and Kirkwood 1969), problems with volatility may be encountered

when esters are used near susceptible non-target plants (Klingman et al. 1975, QueHee and Sutherland 1975). Amines pose virtually no volatility problems (Klingman et al. 1975). Other formulations used to a much lesser degree include emulsifiable acid and mineral salts. Surface active agents (surfactants) are used in conjunction with herbicides for many purposes (sticking, wetting, detergency, etc.), but they are ultimately used to increase the absorption of foliar applied herbicides.

Many authors have discussed the breakdown and persistence of 2,4-D. Bell (1956) and Crosby and Tutass (1968) discuss the rapid decomposition of 2,4-D under ultraviolet light. Klingman et al. (1975:76) list the persistence of biological activity as one month or less for 2,4-D. Lavy et al. (1973) found that essentially all the 2,4-D applied at various depths in the soil had dissipated within five months in Nebraska, while Wilson and Cheng (1976) found small quantities (4 percent) remaining in Washington soils 6 months after spraying. Wilson and Cheng's results were from a very dry year, and 2,4-D persists longer in dry soil (Brown and Mitchell 1948). Brown and Mitchell also found increasing temperatures and increasing amounts of organic materials in the soil to decrease the persistence of 2,4-D in the soil. Microorganisms assist in the breakdown (Newman et al. 1952) and this was shown by the longer persistence of 2,4-D in autoclaved soil (Brown and Mitchell 1948). Morton et al. (1967) have shown that 2,4-D also has a very short half-life in green tissue (two weeks in silver beardgrass (Andropogon saccharoides), little bluestem (Schizachyrium scoparius) and dallisgrass (Paspalum dilatatum)), and virtually disappears after 16 weeks.

The ultimate effect of an herbicide on plant growth is also dependent on the size of the spray droplets. In recent studies, it has been shown that small herbicide spray-droplets have a greater effect on plants than

large spray-droplet sizes. Small (< 0.1 mm diameter) spray droplets of 2,4-D reduce soybean seed production significantly more than large (< 0.3 mm diameter) spray droplets (Ennis and Williamson 1963). Using an insecticide (Malathion), Polles and Vinson (1969) found increased penetration of the leaf cuticle with smaller droplet sizes and speculated that it was due to the greater insecticide surface area involved for leaf absorption when constant volumes of spray were used. Way (1969) found that small droplets (100 microns) had a greater visible effect on lettuce (Lactuca serriola) using 2-methyl-4-chlorophenoxyacetic acid (MCPA) than large droplets (500 microns). Sunflower also showed greater epinasty when sprayed with small droplet sizes of 2,4-D (100 microns) than with large droplet sizes (200 and 400 microns) (McKinlay et al. 1972). They speculated that larger droplets killed the leaf cells directly under them, thus reducing translocation. The total leaf area contacted by a given volume of herbicide would also be greater with smaller droplets. In the study by McKinlay et al., six times as much active ingredient was needed for a given effect using 400 micron droplets as compared to 100 micron droplets.

Increased volatility of small droplets of esters of 2,4-D (QueHee and Sutherland 1975) and drift of small spray droplets are two problems of spraying with small droplet sizes. Volatility can reduce the intended effect of the spray and/or cause damage to adjacent desirable flora. Small spray particles drift a greater distance than large particles and can also cause damage to adjacent flora. Reimer et al. (1966) estimated that a 300 micron droplet falling 50 feet (15.2 m) with a 5 mph (8.1 kph) wind would drift about 100 feet (30.5 m). Brooks (1947) estimated that a 100 micron droplet falling 10 feet (3.1 m) with a 3 mph (4.8 kph) wind would drift 409 feet (124.7 m). Nordby and Skuterud (1974) state that under good

conditions, spray drift of up to 6 percent of the volume can be expected, and half of this would be within a strip 10 m from the end of the tractor-pulled boom. Under most spray conditions, they estimate drift to amount to 1.4-37 percent of the spray volume applied. With drift this prevalent, and the effects of smaller droplet sizes significantly increasing the effect of spray, drift can have an effect on adjacent vegetation and therefore on wildlife.

#### Effects of 2,4-D on Wildlife

Spray drift can affect wildlife either directly or indirectly. Since the publication of Silent Spring (Carson 1962), direct effects of pesticides on wildlife (not just through spray drift) have been widely publicized. Direct effects might come about due to an animal ingesting or coming in contact with a pesticide. In his article assessing the hazards of chemical brush control, Norris (1971:720) concludes "I am satisfied after evaluating the toxicity of 2,4-D, amitrole, 2,4,5-T and picloram that the proper use of these herbicides will not normally result in either an acute or chronic hazard to nontarget organisms on forest lands." At normally applied rates, then, 2,4-D appears relatively safe.

Acute oral toxicity ( $LD_{50}$ ; single dose which is lethal to 50 percent of the animals tested) of the various formulations of 2,4-D range from 300 to 1000 mg/kg (Klingman et al. 1975). Tucker and Crabtree (1970) found  $LD_{50}$  values of 472 mg/kg for pheasant (Phasianus colchicus), 668 mg/kg for coturnix (Coturnix coturnix) and pigeon (Columba livia), and 1000 mg/kg for mallard (Anas platyrhynchos) for 2,4-D technical acid. Mallards given the amine base showed an  $LD_{50}$  of about 2000 mg/kg. Heath et al. (1972) lists the  $LC_{50}$  (lethal concentration in a 5-day diet to kill 50 percent of the animals tested) for bobwhite of all formulations of 2,4-D as greater than

5000 ppm. Another study with bobwhite found an  $LC_{50}$  of 38,000 mg/kg for adult birds and 28,000 mg/kg for young birds (Fish and Wildlife Service 1960). These levels are considerably above those that would be normally encountered by organisms in the wild.

Eggshell thinning has been shown to occur following oral doses of 2,4-D (Haegele and Tucker 1974). Coturnix given a single oral dose of 250 mg/kg produced eggshells which were 50 microns thinner than the normal 200 micron-thick shells of control birds for two days following treatment. Likewise, mallards given 1500 mg/kg produced eggshells that were 30 microns thinner than the normal 330 micron thickness for two days following treatment. Eggshells produced more than two days after treatment were normal in thickness. Chickens given 150 mg of 2,4-D/kg of food showed no adverse effect on egg production, egg or yolk weight, eggshell thickness, or hatchability or growth rate of progeny (Whitehead and Pettigrew 1972). They concluded (concerning 2,4-D) saying "...at the still lower dose rates more likely to be encountered in normal agricultural practice, significant impairment of production is unlikely to occur."

One reason for the short term effect of 2,4-D found by Haegele and Tucker (1974) is the rapid elimination of 2,4-D by animals. Levey and Lewis (1947) found that 60 percent of the phenoxyacetic acid fed to rabbits showed up in excreta within 6 hours of feeding, and 96 percent of it was excreted in 24 hours. Similar results were found with rats (Khanna and Fang 1966) and mice (Zielinski and Fishbein 1967). In sheep (Clark et al. 1964), over 97 percent of an orally administered dose (4 mg/kg) was excreted unchanged in 72 hours.

Recently, teratogenic effects have been found in 2,4,5-T-fed mice. Significant increases of cystic kidney and cleft palate were observed when



mice in day 6 of pregnancy were given 113 mg/kg of 2,4,5-T (Courtney et al. 1970). However, 2,4,5-T did not produce teratogenic effects on chick embryos (Strange and Kerr 1976). Embryotoxicity did occur in rats using 2,4-D, but teratogenic effects did not appear even at levels approaching the LD<sub>50</sub> for rats (Schwetz et al. 1971). Khera et al. (1971) indicated that 2,4-D may cause teratogenic effects (skeletal malformations) in white Wistar rats at high dosage levels. These results, however, were preliminary. In summary, 2,4-D poses little direct hazard to wildlife.

#### Effects of 2,4-D on Plants

Herbicides may influence wildlife indirectly, through habitat change. Effects may differ in differing areas. Martin (1965) found a slight increase in bobwhite populations after a post oak-black oak woodland in Oklahoma was sprayed with 2,4-D and 2,4,5-T. High populations of native grasses and forbs (Ambrosia psilostachya was common) followed the spraying. Forbs and grasses invaded the understory opened up by the dying oaks. When range land consisting of forbs and grasses is sprayed with 2,4-D, forbs are generally eliminated. This has been shown to cause declines in food for pocket gophers (Thomomys talpoides), causing population reductions of approximately 90 percent (Keith et al. 1959, Hull 1971). Johnson and Hansen (1969) found deer mouse (Peromyscus maniculatus) populations were affected very little by 2,4-D treatment of range lands in Colorado. Species dependent upon forbs decreased while species dependent on grasses increased: pocket gopher and least chipmunk (Eutamias minimus) populations declined while montane vole (Microtus montanus) populations increased.

Use of 2,4-D for brush control for livestock can affect many species. Spraying in large blocks has been shown to reduce sage grouse (Centrocercus urophasianus), while strip spraying of sagebrush did not affect use of an



area by adults (Carr and Glover 1970). Young grouse (chicks) were not found in any sprayed areas. Southwood and Cross (1968) found a significant decrease of arthropod numbers and species in sprayed areas in England. Arthropod numbers decreased by more than 2/3, causing a reduction in juvenile survival in partridge (Perdix perdix). MCPA and 2,4-D were the herbicides used. Brewers sparrow (Spizella breweri), dependent on sagebrush for nesting cover, decreased with sagebrush control (Best 1972). He also speculated that the decrease in floral diversity due to 2,4-D would probably cause a reduction in the number and/or species of insects present which Brewers sparrows utilize at certain times of the year.

Most of the native species important as bobwhite food in Kansas listed by Robel (1963) are weeds associated with agriculture and disturbed areas. Nearly all of these are very susceptible to 2,4-D (U.S. Department of Agriculture 1974). Bobwhite, as well as many other wildlife species, utilize these plants in agricultural areas and in areas adjacent to agriculture. Herbicide drift into adjacent areas can occur when spraying weeds in agricultural areas, affecting quail food sources. Very little work has been done on this problem.

Sunflower and ragweed, whose seeds are important food sources for bobwhite in Kansas, are very susceptible to 2,4-D. Throughout much of the bobwhite's range, seeds of common ragweed are one of the most consumed fall and early winter foods (Martin et al. 1951:421). Robel (1963) found ragweed to be the second most important fall (November and December) food in Kansas. By volume, ragweed was the most utilized seed in Virginia (Baldwin and Handley 1946) and Tennessee (Cody 1944), second in Kentucky (Barbour 1951) and Nebraska (Damon 1949), third in Missouri (Korschgen 1948), seventh in the southeastern United States (Davidson 1942), and very important in Oklahoma

(Bird and Bird 1931, Baumgartner et al. 1952), Ohio (Schultz 1949), and Illinois (Barnes and Klimstra 1964, Ellis et al. 1969). In addition, ragweed was highest in frequency of occurrence and volume in disturbed areas in Georgia (Brunswig and Johnson 1973). Other species of ragweed (A. bidenta, A. trifida, and A. psilostachya) can also be locally important food sources to quail (Korschgen 1948, Baumgartner et al. 1952, Wagner 1949, Lehmann and Ward 1941). The effects of low levels of 2,4-D (such as those due to drift) on ragweed seed production are not known.

Where the ranges of sunflower and bobwhite overlap, sunflower seeds are also an important food source. Sunflower seed was found important in Kansas (Robel 1963), Nebraska (Damon 1949), and Oklahoma (Bird and Bird 1931, Baumgartner et al. 1952). Sunflower can also be locally important in Texas (Lehman and Ward 1941) and Missouri (Korschgen 1948). The effect of drift-like levels of 2,4-D on subsequent seed production of young sunflower plants is unknown.

There have been numerous studies concerning the effect of low levels of 2,4-D on plant growth. Weaver (1946) concluded that 2,4-D delayed the onset and decreased the amount of seed production in red kidney beans and soybeans. He also found that low levels of 2,4-D increased the diameters of hypocotyls and first internodes, increased the weight of primary leaves, and decreased the weight of trifoliolate leaves. In a similar study, Weaver et al. (1946) found significant reductions in soybean seed production when the plants were treated in the flowering or early pod stage with 0.5 lb/acre (0.56 kg/ha) of 2,4-D. Spraying when plants were 6-8 inches high did not significantly reduce yield, however younger plants showed greater visible response (epinasty, etc.) than plants treated at a later stage. Baker (1969) found similar results using DSMA and MSMA on cotton.

Slife (1956) found that young (2-4 leaf stage) ragweed plants were more susceptible to 2,4-D than older plants. A rate of 1/8 lb/acre (0.28 kg/ha) of 2,4-D did not control subsequent forb (predominantly ragweed) seed production in Michigan corn fields, while 1/3 and 1/2 lb/acre (0.37 and 0.56 kg/ha) were effective (Jameson and Wheeler 1951). They did not state the stage of growth of the ragweed, but corn was 6-12 inches high at the time of spraying. Peters and Lowance (1970) showed that a decrease in ragweed seed production occurred when plants were sprayed with 0.56 or 1.12 kg of 2,4-D amine/acre. Control was enhanced when younger ragweed plants (15-20 cm) were sprayed. Soybeans sprayed with sublethal (0.015 and 0.150 kg/ha) levels showed non-significant differences from controls when seed yields (kg/ha) were compared (Basnet et al. 1972). Treated plants produced fewer pods, but this was offset by an increased weight/100 seeds ( $P < 0.05$ ). Plants were sprayed at one-tenth bloom stage.

Many authors have studied subsequent germination of seeds from treated plants, and it seems that germination is only affected when plants are sprayed at the seed-filling stage. For example, Rojas-Garciduenas and Kommendahl (1960) found that pigweed (Amaranthus sp.) plants sprayed with 2,4-D at the time of flowering produced seeds that germinated more rapidly than non-treated seeds. Evans et al. (1963) found that amitrole, DMPA, and dalapon seriously reduced seed production and subsequent germination in medusahead (Elymus caput-medusae) when applied at flowering time. Germination, seed production, and weight per seed were significantly reduced when curly dock (Rumex crispus) was sprayed at anthesis (Maun and Cavers 1969). Herbicides are translocated into the seed when photosynthate is being transported into the seed, but the herbicides are not mobile when the "sink" (seed) develops if they are applied before flowering (Thompson and Egli 1973).

Male sterile flowers have also been shown to be induced by 2,4-D when applied before and during flowering (Rehm 1952). Smith et al. (1946) and Grigsby (1945), using 2,4-D, prevented pollen shedding in ragweed. The levels of 2,4-D used, however, were very nearly lethal to the plants. Grigsby (1946) severely reduced seed production in ragweed by spraying with 250 ppm of 2,4-D at the stage just preceding flower stalk elongation. He found 500 ppm was sufficient to kill young plants and to stop pollen production in older plants.

Several authors have considered the problem of spray drift and subsequent seed production in susceptible plants. Greenshields and White (1954) and Greenshields and Putt (1958) simulated spray drift on sweet clover (Trifolium sp.) and cultivated sunflower, respectively, using both amine and ester formulations. These plants were kept downwind of a 2,4-D spray operation. Seed production was delayed and seed quality was reduced in sweet clover when exposed to 2,4-D drift just prior to flowering. Cultivated sunflowers planted 12 May and exposed to drift on 26 June showed reduction of seed production (19 percent) even at distances of 120 rods (603.5 m) from the spraying. Wind velocity was 9-12 mph (14.5-19.3 kph) and a spray pressure of 30 psi was used. Reduction in seed yield was due to small head size, rows of empty seeds in the heads, and lighter weight of seeds. Betts and Ashford (1976) found that while 2,4-D did not reduce subsequent germination of turnip rape seeds from plants exposed to drift before flowering time, seed yield was significantly reduced. No work of this kind has been done with native sunflowers or with very low levels of 2,4-D on ragweed. Very little work has been done on the quality of seeds (protein content, caloric content, etc.) produced by plants subjected to spray treatment.

## METHODS AND MATERIALS

### The Study Area

Sunflower and ragweed have been shown to be very important to bobwhite in Kansas and were therefore the species chosen to study the effects of low levels of 2,4-D on subsequent seed production. High densities of sunflower and ragweed were needed for the study, and these were located on the north-eastern corner of the Fort Riley Military Reservation, 11 km southwest of Manhattan, Kansas. The native vegetation of this area of the western edge of the Flint Hills is prairie. Johnson and Robel (1968) list important plant associations. Although some cultivated crops are grown in the Flint Hills, the primary agricultural practice is grazing livestock.

Because large numbers of sunflower and ragweed plants could not be found in the same area, two study areas were chosen: one with high densities of sunflower and one with high densities of ragweed. The sunflower area was approximately 3 1/2 km north-north-west of the Manhattan Municipal Airport USGS Bench Mark. The area was 150 x 9-m in size and was bounded 20 m on the south by a wind break of primarily Osage Orange (Maclura pomifera) and hackberry (Celtis occidentalis), on the east by a cultivated field of sorghum, and on the north and west by an open field characterized by grasses and other herbaceous cover.

This area was part of a sorghum food-plot program for wildlife carried on by Fort Riley personnel. Sunflowers invaded when the sorghum plot was discontinued. Controlled fires frequently burned the area to minimize woody vegetation. Controlled fires burned the area in mid-March 1976 and in spring 1973.

Soil in the sunflower area is Wymore silty clay loam, with 0-1 percent slopes. This soil is characterized by a high water-holding capacity with a