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Lee, Richard Dalton

THE INFLUENCE OF GIBBERELLIC ACID ON THE PHYTOTOXIC RESPONSE OF FLUAZIFOP-BUTYL IN JOHNSONGRASS (SORGHUM HALEPENSE (L.) PERS.)

The Ohio State University

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THE INFLUENCE OF GIBBERELLIC ACID ON THE PHYTOTOXIC
RESPONSE OF FLUAZIFOP-BUTYL IN JOHNSONGRASS
(SORGHUM HALEPENSE (L.) PERS.)

DISSERTATION

Presented in Partial Fulfillment of the Requirement for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

by

Richard Dalton Lee, B.S., M.S.

****

The Ohio State University

1984

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INTRODUCTION

The influence of gibberellic acid on herbicidal phytotoxicity was first reported with 2,4-D (2,4-dichlorophenoxyacetic acid) and indicated that gibberellic acid application could alter the translocation pattern of the applied herbicide (7,8). Similar results were obtained using bean plants treated with 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) and gibberellic acid in that gibberellic acid increased accumulation of 2,4,5-T in the young shoots and decreased accumulation in roots and nutrient solution (14,15). Studies with naptalam (N-1-napthylphthalamic acid) and gibberellic acid in wheat have shown that gibberellic acid stimulated naptalam uptake from the nutrient solution (43). Similarly, pre-treatment of bean plants with gibberellic acid resulted in greater absorption of naptalam (44). In their study of gibberellic acid-enhanced phytotoxicity of bentazon (3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide) and glyphosate (N-(phosphonomethyl) glycine), Sterrett and Hodgson (209) reported that the enhanced phytotoxicity to Canada thistle (Cirsium arvense (L.) Scop.) may be due to changes in acropetal translocation and accumulation in leaf sinks.

Fluazifop-butyl (butyl-2-(4-(5-trifluoromethyl-2-pyridyloxy)phenoxy) propionate), a recently developed herbicide, is principally used in the post emergence control of annual and perennial grasses
in broadleaf crops. When applied post emergence, the herbicide moves throughout the plant in both the xylem and phloem and accumulates primarily in meristematic regions where it interferes with ATP production (183,193).

Johnsongrass (Sorghum halepense (L.) Pers.) is the principle weed of cotton and sugarcane, as well as orchards, soybeans, and sorghum in the continental United States and Hawaii (87). In 9 of the 11 Southern states, johnsongrass ranks among the ten most costly weeds, irrespective of crops (144).

It was the aim of this research to determine if more effective control of johnsongrass could be obtained through gibberellic acid enhanced herbicidal response of several new post-emergence graminicides. Also to determine what or where the influence of gibberellic acid might be with regards to absorption and translocation.
History of the Plant

Johnsongrass, a vigorous perennial, was early reported to be native to the Mediterranean region from the Madeira Island to Asia Minor and southeastern Europe. Later, authorities stated that johnsongrass was indigenous only to the Mediterranean region (87,150). McWhorter (150) states that it is difficult to establish the presence of johnsongrass in the United States prior to 1875, because of the large number of names used for this species. At least eight different Latin names have been used for johnsongrass and over 40 common names. The names most frequently used include guinea grass, false guinea grass, new guinea grass, true guinea grass, Samoa grass, Cuba grass, Egyptian grass, Means grass, Meanie grass, Alabama guinea grass, Georgia guinea grass, evergreen millet, Arabian millet, Egyptian millet, green valley grass, Australian millet, Morocco millet, Arabian evergreen millet, bankruptcy grass, St. Mary's grass, and Syrian grass, with the most common ones being guinea and Means grass.

The proposed date of introduction into the United States was based upon a bulletin written by C. R. Ball in 1902. He writes that johnsongrass was introduced into this country from Turkey about the year 1830 as a result of a request sent to Governor Means of South Carolina from the Sultan of Turkey for someone to come and instruct
Turks in raising cotton. When the man returned he brought with him seeds of several plants from the area, among them was johnsongrass. About 1840 Colonel William Johnson, the owner of a large plantation near Selma, Alabama, visited South Carolina and returned with seed which he sowed on his plantation (11). McWhorter (150) found that johnsongrass was probably growing at several locations in the United States in the early 1800's. In fact, the earliest story regarding introduction of johnsongrass was found in an unpublished manuscript which claimed that it was introduced in a contaminated shipment of hemp seed from Egypt soon after the Revolutionary War (155).

The use of the name "johnsongrass" as a common name on a national basis resulted from two letters written. One was written by Herbert Post of Selma, Alabama, in 1874 to George Vasey, an employee of the U. S. Department of Agriculture in Washington which was published in the Monthly Report of the U. S. Department of Agriculture. The second letter was written by John Haralson of Selma, Alabama in 1880 to D. L. Pharis of Woodville, Mississippi, identifying johnsongrass as the common name used in the area. These two publications gave enough of an exposure of the name "johnsongrass" such that the common name of "johnsongrass" was generally accepted by the mid-1800's (150, 161).

Description of the Plant

Johnsongrass is described as an aggressive perennial grass with well developed creeping and rooting, scaly rhizomes. Culms slender to robust, erect, mostly simple, 0.5 to 3.5 m tall and up to 2 cm wide near the base. Leaf blades linear up to 90 cm long and 4 cm
wide, alternate, smooth, or rough on the edges, many-nerved, with conspicuous midribs. Sheaths ribbed, smooth, or often hairy within the junction of the blade. Ligule is short and papery. Inflorescence is a large and more or less open panicle 10 to 60 cm long and 5 to 25 cm wide, with the lower branches up to 8 cm long in small inflorescences and up to 25 cm long in large ones, pyramidal, purplish and hairy. Panicle branches slender, often somewhat pendulous, usually naked for 2 to 5 cm from the base, divided upward. Racemes fragile, 1 to 5 noded up to 2.5 cm long, pedicels densely ciliate. The spikelets are in pairs, one spikelet being sessile the other pedicellate, except for the terminal groups which are in triplets 4.0 to 6.5 mm long, more or less elliptic-lanceolate, almost glabrous to densely hairy, awned or awnless. Glumes coriaceous, lower 7 to 12 nerved, with the wings of the keels ending in minute teeth, forming with the pointed apex a 3-toothed tip: upper glume not 3-toothed. Lemmas ciliate, lower 3 to 5 mm long, upper 3 to 4 mm long, minutely bilobed with usually a 10 to 16 mm long awn. Stamens three. Grains oblong ovate, 2 to 3 mm long, reddish brown, glossy, marked with fine lines on the surface. Pedicellate spikelets male or neuter, 4 to 7 mm long. Sessile spikelets distinctly articulate with the subtending joints and are readily deciduous with the accompanying pedicellate spikelets and superior joints (45,87).

The johnsongrass plant is very similar to grain sorghum and has been thought to have originated from the cultivated species. It has been shown that it will cross pollinate with sorghum to create new strains with various characteristics of both species (68,166).
Ecotypical differences in johnsongrass have been found. An ecotype has been defined as a genotypic variant of a species which arises as a response to a particular habitat (166). McWhorter, et al. (148,159) found, while studying ecotypes from the United States and several countries, that plant heights vary nearly 2-fold. Primary culm production ranges from 5.0 to 9.3 and secondary culm from 7 to 2\textsuperscript{h} per plant system. Leaf blades were found also to vary in length from 31 to 59 cm and in width from 1.7 to 3.4 cm. Seed production also varied, in individual panicles, more than four-fold. Other workers have indicated that the time required for flowering to occur is also an ecotypical response (24,25,159,226). Burt (24), studying 12 different ecotypes, found that they grew different depending upon the environment of origin. Plants from the more northern latitudes consistently flowered earlier than plants obtained from the more southern latitudes.

Growth Factors and Characteristics

Johnsongrass can grow on a variety of sites: on arable land, waste places, roadsides, and field borders. It occurs extensively along irrigation canals and at the edge of irrigated fields. Its general distribution in these areas is the result of water movement of the seeds, which readily fall from the head when mature (87). An individual seedhead may produce 300 to 400 seeds, or about 28,000 seeds per single plant. In severe infestations this is equivalent to 10 bushels of seed per acre (91,161). The seed of johnsongrass has been reported to remain viable in the soils for several months. In looking at a 2.5 year sample of a 50-year study, it was found that
johnsongrass seed had high viability, 82% (55). Not only does johnsongrass have high viability in the soil, but it also exhibits dormancy (77,78,217). Rojas and Castro (191), in laboratory tests, found that recently harvested seed achieved 12% germination; the figure increased to 50% at 9 months and 85% (the maximum) at 13 months. Keeley and Thullen (117) also reported that seed planted one month after collection had 30% germination, but increased to 90% after six months.

Dormancy has been reported to be largely imposed by the relative degree of mechanical restriction afforded by the seed coat (217). Removal of the glumes has given 95% germination in some experiments with dormant seed (77,78). It is believed that seeds from the current season have low germination in the field but seeds which have lain in the soil for 1 year will germinate very readily.

Keeley and Thullen (117) presented information which showed that johnsongrass seedlings will begin to emerge when soil temperatures at 5 cm reach 14 to 15 C. They also stated that when soil temperatures at 5 cm averaged 24 C for 2 weeks after planting, 18% and 40% of the seedlings emerged within 1 and 2 weeks after planting, respectively. This is supported by Horowitz (89) who indicated that johnsongrass seeds will begin germination at 19 C and reach a maximum at 39 C.

Evetts and Burnside (56) indicated that johnsongrass exhibited the fastest shoot growth rate of any of nine plant species studied. The rhizome spur has been reported to begin growth 2 to 3 weeks after shoot emergence, in some situations (146,166), but not until 5 to 7
weeks after emergence, in other reports (6,89,117,134,175). Horowitz (89) found that the maximum temperature for rhizome formation was between 15 to 20 C.

After formation of this spur, rhizome growth was relatively slow and apparently subordinate to the much more rapid top growth, which was slow up to the time of rhizome initiation (166). About the same time the rhizome spur developed, the johnsongrass plant started tillering, which proceeded throughout the growing season (89,117).

Flowering begins 7 weeks following the emergence of the seedling and will continue for the rest of the growing season (89,117,146,166). After the start of blooming, rhizome production increases rapidly, whereas the growth rate of leaves decreases. No causal relationships between flowering and rhizome formation rates has been found to occur (89). McWhorter and Stroube (161) indicated that nearly 90% of the annual rhizome production occurred after flowering. During the early stages the rhizome functions primarily as a storage organ and carbohydrate buildup is rapid (6,175). Hull (94) says that flowering and early grain production appear to have low priority for assimilates produced in most portions of johnsongrass shoots, and it is not surprising that assimilate translocation into rhizomes is maximal during more advanced growth stages. He further states, as a result of a $^{14}$C-labelled assimilate study that, the marked decline in retention of the $^{14}$C assimilates by leaf and stem tissue during flowering suggests that these tissues may serve as storage sites for photosynthates until sufficient rhizome tissue has been produced. At flowering, secondary rhizome growth is extensive, offering ample
space for carbohydrate storage. The rapid increase in secondary rhizome growth between boot and flowering stages may occur through the utilization of assimilates stored in culms and leaf tissue.

Keeley and Thullen (117) reported the total fresh weight of plants 6, 9, and 12 weeks after planting to show what is going on in the overall development of the johnsongrass plant. The shoots, at 6, 9, and 12 weeks, accounted to 70, 66, and 54% of total fresh weight, respectively. The roots accounted for 16, 9, 6%, respectively for the three time periods, and the rhizome accounted for 13, 25, and 40% of the total fresh weight, at 6, 9, and 12 weeks, respectively. These results would demonstrate the shift of total carbohydrate content of the plant over time from shoots and roots to the rhizomes.

McWhorter and Jordan (160) reported that maximum growth rate of rhizomes was achieved at 32 C and minimal when the temperature reached 40 C. Johnsongrass plants are capable of producing large numbers of rhizome. A single plant can produce 70 meters of rhizome in one growing season (166). This high rate of growth has been described as follows: a 3-inch rhizome planted at Stoneville, Mississippi produced 4 feet of rhizome in 40 days, 70 feet in 80 days, and 150 feet in 120 days (156), which could result in as much as 33,600 kg/ha per year (207).

Rhizomes classified as primary are those alive in the ground at the beginning of the growing season in the spring; as secondary, those which arise from primary rhizomes, come to the surface and there form crowns, thus producing new plants; and as tertiary, those which develop from the base of the crown at flowering time (28,216).
It is the tertiary rhizomes which become the primary rhizomes the next year.

McWhorter (151), studying rhizome production in three different soil types, found that rhizomes production was twice as great in sandy-loam as in clay soil, 2458 g/m² and 988 g/m², respectively, and approximately 10% less in silty clay loam, 2234 g/m², than in the sandy-loam. He reported that 80% of the rhizomes were in the to 7.5 cm of the clay soil, while 80% of the rhizomes were located in the top 12.5 cm of the sandy-loam soil. In a clay soil 60, 30, and 10% of the total subterranean weight was found in 0 to 15 cm, 15 to 30 cm, 30 to 45 cm soil layer, respectively (90).

Following the completion of the johnsongrass plant's life cycle, the shoot of the plant deteriorates in late fall and the process of overwintering occurs. It has already been reported concerning the viability of johnsongrass seed, which is one of the ways johnsongrass overwinters (55). Stoller (212) observed that rhizomes at depths of 20 cm or more survived the winters in Urbana, Illinois. These rhizomes were killed at soil temperature below -9 C, which is similar to the results of McWhorter (151) in studies using germination chambers. He found that johnsongrass rhizomes could withstand temperatures down to -3 C for 24 hours in soil before 100% of the rhizome died. Stoller (212) concluded from this that low soil temperatures limit the northern range of johnsongrass in the United States by killing rhizomes. McWhorter and Baldwin (156) concluded that there is increasing evidence that johnsongrass continues to adapt, grow, and reproduce in less temperate climates. They cited reports where
johnsongrass had overwintered in Canada as well as in several counties in New York state.

As soil temperatures warm in the spring, buds situated at nodes on the rhizomes become active and produce vigorous johnsongrass plants. Seeds also germinate at this time but give rise to less vigorous seedlings. This is because the plants arising from the rhizome have the carbohydrate reserves of the rhizome to draw upon during the first two weeks, while those arising from seeds have only the limited reserves in the seed (146).

Hull (94) argued that johnsongrass rhizomes buds fail to demonstrate true physiological dormancy but that correct environmental conditions is all that is required for buds to sprout. It has been shown that, although dormancy is not involved in sprouting, rhizomes are under the control of apical dominance. There is a suppression of buds, from germinating, basipetally to the apical end, with the degree of suppression decreasing as the distance from the apex increases (17,94). Studies dealing with bud activity on rhizomes have shown that the optimal air temperature for bud germination was approximately 30 C and that sprouting was nil at 10 C and slow below 20 C (89,94). Horowitz (90) demonstrated that there was no difference in percent sprouting with regards to depth at which the rhizomes had grown. Approximately 45% sprouting occurred in rhizomes recovered from 0 to 15 cm, 15 to 30 cm, and 30 to 45 cm depths. McWhorter (151) found that more plants emerged from short rhizomes (76 mm) than from long rhizomes (152 mm) when planted at a depth of 7.6 cm. He also found that germination of buds was less for rhizomes
with six or more buds than those with one to four buds per rhizome segment.

**Losses Due to Johnsongrass**

Johnsongrass has become established as a formidable weed in most of the agricultural areas of the world in which it is adapted. It seems best adapted to the warm, humid, summer-rainfall areas in the subtropics and not so well adapted to areas which are strictly tropical (87). It is the principle weed of cotton and sugarcane as well as orchards, soybeans, and sorghum in the continental United States and Hawaii (87). McCormick (114) states that johnsongrass ranks among the ten most troublesome weeds in cotton and corn in the southern United States and among the ten most costly weed irrespective of crop in 9 of the 11 Southern states. Regarding cotton and losses resulting from johnsongrass infestations, a survey conducted since 1976 shows that johnsongrass is present in 70 to 80 percent of all cotton fields from mid-August to early September in the delta area of Mississippi, Louisiana, and Arkansas (156). It has also been reported as a major noxious weeds in cotton grown in California (116). Infestations of johnsongrass have been reported to result in losses in yield of cotton ranging from 70 to 80%. It has been shown that 20 johnsongrass shoots per m² could be expected to reduce yields of seed cotton 20%, and densities exceeding 75 shoots per m² could result in losses exceeding 75% (118). The range of reduction in yield in corn production due to infestations of johnsongrass was 19 to 70% (95,103,161).

In Louisiana, johnsongrass thrives under the cultural conditions
suitable for sugarcane and is the most noxious weed. The banks of drainage ditches serve as the primary source for johnsongrass dissemination. These ditches occur at 12 to 20-row intervals in sugarcane fields and occupy approximately 10% of the total field area (163).

As was stated earlier, johnsongrass is a serious problem in the production of soybeans. Reports of heavy infestations of johnsongrass have indicated reduced soybean yields ranging from 23 to 83% (64, 155, 157, 158, 161). McWhorter and Hartwig (158) stated that the apparent seed weights from combine harvesting were often higher than hand-harvested weights because of contamination, in a severe johnsongrass infestation, by johnsongrass seed and trash. McWhorter and Anderson (155) indicated that foreign material in soybean seed samples was about 0.8% with 100% johnsongrass control and nearly 6% with no control. A minimum of about 95% johnsongrass control was needed to avoid more than 1% foreign material in harvested soybeans. Deduction for excess foreign material in soybeans begins at 1% so that U.S. grade #1 soybeans were obtained when johnsongrass control was 95% or better. At least 70% control was required to avoid deductions from gross harvested weights caused by seed moisture levels exceeding 13%. Uncontrolled johnsongrass resulted in a predicted soybean grade of 4.1%. Soybeans were reduced nearly one level for each 30% reduction in the level of johnsongrass control obtained.

Johnsongrass may interfere in crop production in other ways than through competition or seed contamination. Abdul-Wahab and Rice (1) reported that decaying johnsongrass rhizomes or leaves in the soil
inhibited the germination and subsequent development of most of the seedlings of seven weed species studied. They also found that live johnsongrass roots and rhizomes exude a toxin (p-hydroxybenzaldehyde) or toxins that are inhibitory to germination and growth of several weed species. In other studies, inhibition of barley (*Hordeum distichum* L.) seedling growth was proportional to the concentration of dry rhizomes incorporated in the soil (92). Similar results were found with water or ethanol extracts of two soils in which rhizomes had decayed for various periods (62,92). Studies conducted in North Carolina indicated an adverse relationship between soybean seedling dry weight and percent of dry, decayed rhizomes in the soil. Fresh and dry weights of soybean plants were reduced by diluted fresh rhizome extract used for irrigation once a week for three weeks (135). It was concluded that johnsongrass rhizomes, living or decaying in the soil, exude, contain, or produce substances that exhibit allelopathic characteristics to soybean growth.

**Practices for Controlling Johnsongrass**

Cultural and mechanical control of johnsongrass are practiced mainly to control seedlings and deplete seed populations already in the soil, to kill existing shoots and their attached rhizomes, to prevent the formation of new rhizomes, and to prevent seed production. Several systems have been suggested to achieve these goals. Some of the most effective systems combine the use of cultural, mechanical, and chemical control methods (39).

Burt and Willard (26) reported that cultural practices are sufficient to control johnsongrass if one season can be devoted to
them. It is recommended that the area be plowed when the johnsongrass is 18 to 36 inches tall, then keep the ground worked so that johnsongrass does not grow more than 8 to 12 inches high during the remainder of the season. Beginning early in the summer is the most important single step in this control. Using this type of summer fallowing reduced rhizome production over 99% when a heavily infested area was thoroughly tilled six times at 2-week intervals (161).

Anderson, et al. (6) reported scattered shoot development even when rhizomes were dried to 25% of their original weight, but drying to 20% killed all rhizomes. It was found that shorter rhizomes dried faster. Therefore repeated tillage, as with a disk harrow in the summer fallow program, would cut rhizomes into smaller pieces, facilitating control. The disadvantage of such a program is that the infested area is out of production for one season and there should be a follow-up program for seedling control. The need for seedling control was demonstrated by McWhorter (146) who found that 13 day seedlings were killed by one clipping. When seedlings were not cut until 20 days after emergence, at which time the rhizome spur had been initiated, they were not killed by eight successive weekly clippings.

McWhorter and Hartwig (157) found that diskings 10 times before planting soybeans on June 5 destroyed 90% of the rhizomes and the soybean yielded 2340 lbs seed/acre. Soybeans planted on May 8, which is a near optimal date for planting in the Delta area of Mississippi, after 4 diskings, yielded only 1200 lbs seed/acre. This delayed-planting method enables the grower to have some use of the land as
well as obtain control of johnsongrass in the process.

Flooding also reportedly controls johnsongrass. McWhorter (152) reported that flooding the soil with 5 to 10 cm of water for 7 to 14 days killed all newly planted rhizomes, as well as those already found in the field. It was found in the greenhouse experiment that it is important to flood immediately after planting. He stated that flooding four-week old johnsongrass plants resulted in a type of induced dormancy.

Chemical control has been and still is considered the most important means of controlling johnsongrass whether in cropland or noncropland. Burt and Willard (26) first reported in 1959 that if johnsongrass was plowed at least one month before frost 70 to 95% control by TCA (trichloroacetic acid) applied at 40 to 60 lbs per acre or by sodium chlorate applied at 100 to 200 lbs per acre could be achieved. The high cost of TCA, due to its high application rates, restricted its use.

Dalapon (2,2-dichloropropionic acid) is effective as a foliage spray for grass control. At rates of 2.0 to 7.5 lbs/acre, dalapon is effective when used in conjunction with a summer fallow program or in a spring treatment when johnsongrass is 8 to 12 inches high, waiting 3 days before plowing and planting (161). Dalapon applications have been reported to erratic control (156). Working with 14 ecotypes of johnsongrass, McWhorter (149) reported average control of different ecotypes following treatment with dalapon varied from 36 to 91%. This was an over-the-top application rate of 10 lbs/acre and would support the claim of erratic control resulting from dalapon usage.
The organic arsenical herbicides MSMA (monosodium methanearsonate) and DSMA (disodium methanearsonate) are also effective in controlling johnsongrass, used particularly in cotton (71,118,161,199), but also in sugarcane (165). Keeley and Thullen (118) and McWhorter (146) observed that DSMA killed johnsongrass foliage in the field, but retreatment was necessary because little of the herbicide apparently translocated into rhizomes.

McWhorter and Baldwin (156) indicate that in conjunction with the arsenicals, trifluralin (2,4,5-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) was introduced. Low rates of trifluralin have been shown to be effective in controlling seedling johnsongrass, but higher rates are required in order to control rhizome johnsongrass (153,154). Standifer and Thomas (208) summarized the results of their greenhouse experiment using trifluralin by saying that johnsongrass seedlings were killed when the first node passed through the treated soil (0.56 kg/ha). Shoots from rhizomes were not killed, but lateral root development was inhibited on the stem portion in contact with the treated soil. Millhollon (165) reported on the effect of the length of the rhizome segment placed in trifluralin treated soil. He found that 93% of the 2.5 cm long rhizome pieces planted in 1.68 kg/ha trifluralin treated soil, whereas only 52% control resulted with 15 cm rhizome pieces placed in the same trifluralin treated soil. The use of trifluralin has been reported to enhance the phytotoxic response of dalapon and MSMA (121,177).

There are several other herbicides which selectively control johnsongrass. In corn, johnsongrass seedlings can be controlled with
butylate (S-ethyl diisobutylthiocarbamate), butylate plus atrazine (2-chloro-4-(ethylamino)-6-isopropylamino-2,4-triazine), alachlor (2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide), EPTC (S-ethyl dipropyl thiocarbamate), and metolachlor (2-chloro-N- (2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide) (85,161,190).

In soybeans, trifluralin, nitralin (4-(methylsulfonyl)-2,6-dinitro-N,N-dipropylaniline), fluchloralin ((N-(2-chloroethyl))- , , -trifluoro-2,6-dinitro-N-propyl-p-toluidine), profluralin (N-(cyclopropylmethyl)- , , -trifloror-2,6-dinitro-N-propyl-p-toluidine), pendimethalin (N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamide), metribuzin (4-amino-6-tert-butyl-3-(methylthio)-as-trazin-5(4H)-one), alachlor, metolachlor, oryzalin (3,5-dinitro-N^I,N^-dipropylsulfanilamide), and chloramben (3-amino-2,5-dichloro-benzoic acid) can be used for seedling johnsongrass control (151, 161).

Glyphosate, a non-selective post-emergence herbicide, has been reported to give excellent control of johnsongrass when applied at the boot stage (34). In recent years two new post-emergence herbicides have been registered for selective johnsongrass control in soybeans, fluazifop-butyl and sethoxydim (2-(1-(ethoxyimino)butyl-5-(2-ethylthio)propyl)-3-hydroxy-2-cyclohexen-1-one) (38).

Probably the most important part of a johnsongrass control program is preventing the spread of seed. All farm equipment, especially harvesting machinery, must be thoroughly cleaned when moving from an infested to a non-infested area (161).
History and Classification of Gibberellins

The discovery of this group of plant growth regulators occurred in the 1920's. A Japanese plant pathologist, Eiichi Kurosawa, went to Formosa to work with Dr. K. Swada at the Central Research Institute of the Formosa Department of Agriculture. His project was to work on methods of controlling the bakanae disease in rice (213). Bakanae, "foolish seedling", disease, was recognized as one of the three most serious diseases of rice (97), is caused by the fungus Gibberella fujikuroi (Saw.) Wollenw. (also known as Fusarium moniliforme Sheld.). Symptoms shown by rice infested with this fungus were an excessive elongation of stems and leaves, resulting in abnormally tall plants which usually fell over due to the spindly stem structure (223).

In 1926 Kurosawa (126) showed that a sterile filtrate of the fungus, which at that time was known as Fusarium heterosporum Ness, which infected rice plants, could also stimulate the growth of rice and grass seedlings. Yabuta, Hayashi, and Kanabe studied the isolation of the active principal from the fungus. They were later joined by Kurosawa who provided the proper fungal cultural which could produce the active principal abundantly in vitro (213).

In 1935 Dr. Yabuta gave it the name of gibberellin on the basis of the scientific name of the fungus (213). Finally in 1939 a small quantity of highly active crystalline material was isolated from the fungal culture filtrates and was given the name gibberellin A (223).

The first work on gibberellins, outside the Orient, was done at the Chemical Corps Biological Laboratories at Camp Detrick, Maryland
in 1950. Shortly thereafter Imperial Chemical Industries, England, started investigating gibberellins also (213). It was not until 1958 that the first chemically pure gibberellin from a flowering plant, Phaseolus multiflorus Lam. (Phaseolus coccineus), was isolated by MacMillan and Suter (140). By 1961 there were 9 gibberellins, 6 from Gibberella fujikuroi and 3 from immature seeds of Phaseolus multiflorus Lam., which had been isolated (215). When Paleg (177) reviewed the subject in 1965 the number was up to 13, but had increased to 29 in just five more years (129). Bearder reported in 1980 that 57 compounds had been isolated and found to fit into the definition of gibberellins and more recently gibberellins number 60, 61, and 62 were identified from seeds (120).

All gibberellins have either 19 or 20 carbon atoms grouped in a total of four or five ring systems. All gibberellins have one or more carboxyl groups. They are abbreviated GA, with the subscript such as GA$_1$, GA$_2$, GA$_3$, and so on, to distinguish them. All could properly be called gibberellic acid, but GA$_3$ (Fig.1) has been studied much more than the others and it is the one that is usually referred to as gibberellic acid (9,196).

Gibberellins exist in angiosperms, gymnosperms, ferns, algae, and fungi, but apparently not in bacteria (196). Audus (9) stated that gibberellin-like substances are not confined to plant tissue since a wide variety of animal tissue (e.g. earthworm bodies, silkworm pupae, chick embryo, and cod fish sperm) have shown to contain substances which promote $\alpha$-amylase production in rice endosperm. Whether these are gibberellins extracted by the animal from their
Figure 1. Chemical structure of GA$_3$ with the accompanying ring designations and carbon numbers.
plant food or whether they are natural animal products that are
not true gibberellins but have some of their characteristic properties
has still to be defined.

The classification of gibberellins has been based upon two major
differences: a) possession of 19 or 20 carbon atoms and b) the
presence or absence of OH groups in positon 3 and 13. The 20-C
gibberellins have carboxyl groups in positions 7 and 18 and some also
have a carboxyl group in positon 20, while others have an aldehyde
group in the latter position. The 19-C gibberellins are mono-
carboxylic acids with the carboxyl group in position 7. They also have
a lactone bridge configuration between carbon 19 and 10. This is in
connection with the loss of the "extra" carbon atom (16,129).
The presence or absence of hydrolysis in positions 3 and 13 seems to
point up a difference between those gibberellins that occur in the
fungus and those which occur both in fungus and in higher plants, or
only in the latter. If a gibberellin occurring in the fungus only has
a single OH, it is always in the 3-positon, whereas gibberellins found
in higher plants always have the OH group in positon 13 (Fig. 1)
(129). It is therefore assumed that two different major pathways for
gibberellin synthesis exist in higher plants one of which also
operates in the fungus. The pathway common to fungi and higher plants
is characterized by an initial hydroxylation in the 3-position. The
other pathway, unique to higher plants, has an initial hydroxylation
in the 13-position.

Biosynthesis, Metabolism, Extraction, and Purification of Gibberellins

In general, it has been found that young developing seeds contain
relatively large amounts of gibberellins. As the seeds mature and their growth slows down there is a simultaneous decline in gibberelin content. Gibberellins are most likely moving out from the young developing seeds (223). Moore and Eckland (167) commented that seeds and fruits, particularly when immature, contain large amounts of gibberellins compared to vegetative organs of established sporophytes. Commonly, the levels of gibberellins in immature seeds are approximately two orders of magnitude greater than in leaves, stems, and roots. For this reason primarily, developing seeds and fruits are popular materials for investigations of the gibberellin of higher plants.

The metabolic process of gibberellin synthesis has been reported to occur in young developing leaves, developing seeds, and root tips (82, 108, 131, 138, 196, 201, 223). Salisbury and Ross (196) stated that the diffusion technique shows that young leaves are the major site of active gibberellin synthesis while mature leaves have little ability to synthesize gibberellins. Roots also synthesize gibberellins in significant quantities, yet gibberellins have little direct effect on root growth, except that they inhibit adventitious root formation (223). The experiments of Jones and Phillips (108) lend support for the conclusion that the apical bud is an important source of gibberellin-like substances which are involved in the control of stem extension growth. They indicated that it is the young leaves of the apical bud, rather than the apical meristem, which serve as sites of gibberellin synthesis. MacMillan (138) identified two specific sites of synthesis, the scute-lum of the germinating barley embryo, and
the chloroplast. Hedden, et al. (82) and Wareing and Phillips (223) have reported that plastids also contain gibberellin-like substances and are capable of carrying out at least some steps in gibberellin biosynthesis.

The biosynthetic pathway involves a series of reactions starting with acetyl-CoA and ending up with GA\(_{12}\)-aldehyde from which the C\(_{20}\) and C\(_{19}\) gibberellins are made (Fig. 2) (201,223). The overall process can be summed up this way, the starting point is acetic acid which is linked, three molecules at a time, to form the intermediate mevalonic acid. It loses one CO\(_2\) forming the five carbon isoprene-like building blocks isopentyl. The next step involves the stringing together of the isopentenyl groups, four of which combine to form the geranylgeranyl pyrophosphate intermediate. This is followed by cyclization to form kaurene and finally a series of oxidations steps, including a contraction of one of the rings, to form the first synthesized 20-carbon gibberellin, and the 19-carbon gibberellins from that (9,131,178).

Echols, et al. (53), in 1981, concluded that knowledge of the regulation of gibberellin biosynthesis remains fragmentary due to the complex nature of the gibberellin pathway and the low endogenous levels of gibberellins in plants. Zeevart and Osborne (236) stated that enzymes involved in gibberellin biosynthesis in different organisms exhibit certain variations which make them more or less sensitive to inhibitors. This would also complicate the findings of a gibberellin biosynthesis regulatory experiment.

Once formed, gibberellins appear to be only slowly degraded,
Figure 2. Biosynthetic steps leading to the formation of gibberellins using several gibberellins as examples.
but they can be readily converted to bound forms (196). Sembder, et al. (201) indicated that the known gibberellin conjugates can be divided into hydroxy-bound GA-O-conjugates (glucosyl and acyl derivatives), carboxyl-bound GA-O-conjugates (glucosyl and alkyl ester), or carboxyl-bound GA-N-conjugates (amino acid derivatives). The most common of these conjugates are the ones in which glucose is bound to a hydroxyl group or carboxyl group of the gibberellin. Conjugation leads to partial or complete loss of biological activity. The potency of gibberellin conjugates, as measured in different bioassays, parallels rate of hydrolytic cleavage and is, therefore, based upon the ability of plant enzymes, and possibly of certain microbial activities, to hydrolyze glucoside, glucosyl, or alkyl ester and amide linkages. Stoddart and Venis (211) state that neither the glucosyl esters nor the O-glucosyl ethers are biologically active per se. Those glucosyl conjugates which do show activity in conventional bioassays are believed to owe their activity to hydrolytic cleavage to the free gibberellin either by plant enzymes or by microbial contamination under non-sterile conditions of the bioassay.

Evidence has indicated that gibberellin glucosides may play a role in controlling the status of free gibberellins within the plant (107). Sembder, et al. (201) stated that gibberellin glucosylation apparently leads to reversible deactivation. Barendse, et al. (12), in an experiment using labelled GA₁ and developing seeds, found that during seed development part of the gibberellin was converted to a "bound" form, while during early germination part of this bound form was reconverted to free gibberellin. Stoddart and Venis (211), in 1981,
indicated that the function of gibberellin conjugates was still an open question. It has been proposed, they continue, that the polar, water soluble conjugates may act as transportable forms which are hydrolyzed to the active free gibberellin in the sink tissue, but currently there is little direct evidence to support this idea.

Other important inactivation processes include 2-β-hydroxylation, which does not appear to be reversible and results in complete loss of or at least greatly reduced biological activity of the gibberellin (223), and the metabolic conversion of highly active gibberellins to other less active gibberellins (196).

Extraction of gibberellins from culture filtrates of Gibberella fujikuroi is by adsorption on activated charcoal. Adsorbed substance are then eluted by ethanolic ammonia or by acetone. Extraction from higher plants poses different problems since the amount of gibberellin present is very low (of the order of 0.5 mg/kg fresh weight) and, in addition, more refined steps for concentration and purification are needed (9). Fresh material can be extracted by acetone/water or methanol/water mixtures of the material can be freeze-dried and extracted with ethyl acetate.

Brenner (20) stated that often the initial purification step after extraction was the partitioning of the plant sample between immiscible solvents. Gibberellins so far isolated comprise acidic and neutral gibberellins, with the acidic gibberellins consisting of free gibberellins and gibberellin glucosides and the neutral gibberellins comprising the glucosyl ester gibberellins. Adsorption column chromatography with charcoal as the adsorbing material, due to its
capacity to deal with a large amount of sample, or with silicic acid, usually have been applied to partially purified gibberellin fractions (235). Partition column chromatography, using Sephadex as the support for the stationary phase, and gel permeation column chromatography are very rarely used for purification of gibberellins (235). The other purification procedure mentioned is counter-current distribution. This procedure depends on the partitioning of mixed compounds between two solvents. Separations are based on differences in partition coefficients. It is generally used at an early stage in purification of plant hormones because it can take a large amount of sample (235).

If the plant hormone can be isolated in sufficient amounts and in pure state, IR, UV, NMR, and mass spectrometry can be used for identification by comparing spectra of unknown and authentic specimens. However, as happens quite often, samples in such quantity and purity cannot be easily obtained in plant physiology studies, therefore, other analytical methods such as paper chromatography, thin-layer chromatography, gas-liquid chromatography, and combined gas-liquid chromatography-mass spectrometry are used for identification of plant hormones (20,29,139,235). Russell (194) indicated that with gas-liquid chromatography-mass spectrometry there are limitations. First, it is only capable of identifying known gibberellins for which chromatographic behavior and spectra are established and, second, it is not applicable to all crude extracts. This has been shown by Crozier, et al. (35) who found it impossible to identify the gibberellins present in crude extracts of vegetative tissue this way.
Brenner (20) reported that until recently analytical procedures for most plant growth substances had to incorporate at least one step involving thin-layer chromatography. Due to the greater sample capacity and resolving power of a newer technique, High-performance liquid chromatography (HPLC), it is suggested that HPLC will replace thin-layer chromatography procedures. The procedure for using HPLC for gibberellin isolation, purification, and identification has been covered by Barendse, et al. (13) and Jones, et al. (105).

Bioassays have been used to determine the biological activity of the different isolated gibberellins. Some of the bioassays involve the use of seedlings, leaf growth, barley endosperm, leaf senescence, and stem apex (9,36,169,203,204). The barley endosperm bioassay is the most common bioassay used and is very sensitive since one of the most striking actions of the gibberellins is in promoting and sometimes inducing germination (9,36). One of the first events in the process of germination is the appearance of the enzyme, $\alpha$-amylase, which metabolizes the reserve starch in the endosperm and releases reducing sugars. This enzyme, which is produced in the aleurone, can be induced to appear by gibberellin treatments of low dosages ($10^{-3}$ ug). This is the basis for the bioassay which is completely insensitive to IAA or other hormones (9). As with the barley endosperm, the other bioassays involve plant responses which give visual results in conjunction with small exogenous applications of gibberellin-like compounds. These responses, which include elongation of stems, delayed senescence, and increased leaf growth, provide a means of readily identifying a known gibberellin response.
Transport of Gibberellins

Gibberellin-like substances occur in the sieve-tube of several plants, e.g. *Vicia faba*, *Taraxacum officinale*, and *Robinia pseudoacacia* (86,122) which indicates phloem transport of gibberellins. It has also been shown that exogenously applied gibberellic acid follows a distribution pattern within the plant typical for substances moving in the phloem (31,143) with the rate of movement being in accordance with phloem movement in these plants. Xylem transport of gibberellin-like substances have been deduced from the presence of gibberellin-like substances in xylem exudates (129). These and other reports (179, 200,223) have given strong evidence for the non-polar movement of gibberellins in both the xylem and phloem. Lang (129) wrote that the emerging picture is that there is a ready transport of gibberellins in the entire conductive system of the plant, proceeding passively with the flow of assimilates or by water plus salts and some other organic compounds.

Experiments conducted by Jacobs (98), Jacobs and Kaldeway (100), and Jacobs and Pruett (101) have demonstrated a polar, basipetal movement of gibberellins in coleus petioles. It was found that when a low "physiological" amount of gibberellic acid was added in donor-agar to excised sections, paralleling the classic auxin transport experiments, the time-course movement of gibberellic acid into the basal receivers was remarkably like that of IAA (101).

The movement of gibberellins over shorter distances, from the shoot tip where it is made to the elongating internodes where it regulates growth, however, is not fully understood. Salisbury and
ross (196) indicate that it almost surely does not involve vascular tissue, because young leaves import but rarely export through either xylem or phloem. Presumably cortex or pith is involved. Drake and Carr (49), studying the transport pathway of gibberellins in oat coleoptile, indicated that the longitudinal transport of gibberellins was non-polar. They also state that the major pathway of gibberellin transport is symplastic, i.e. involves the plasmodesmata.

Another aspect of gibberellin transport is transport into the plant when applied exogenously. Movement of a growth regulator from a solution on the external surface of a leaf to its site of action within the plant begins by diffusion through the cuticle. The rate of diffusion is proportional to the gradient across the cuticle and the magnitude of this gradient depends upon the availability of the applied regulator, the internal concentration of "free" regulator, and the thickness of the cuticle. The availability of the regulator is influenced by its polarity, by the physical properties of the applied solution, and by the morphological characteristics and structure of the cuticle (197). The morphological characteristics of the cuticle have been described by Baker (10). McComb (143) reported that exogenously applied gibberellins penetrate the leaf surface only while the application spots are moist. Ohlrogge, et al. (174), in an experiment which involved feeding $^{3}$H GA$_{1}$ (10$^{-7}$ M) to excised barley leaves for 3 hours, then allowing the leaves to stand in water for an additional 13 hours, found when the epidermis was peeled that it contained less than 3% of the total radioactivity taken up by the leaves through the leaf surface into the mesophyl cells.
It was estimated, from their results, that the mesophyl cells of barley are at least 10-fold more active than epidermal cells in accumulation of gibberellic acid fed through the excised leaves. In Sorghum leaves, the epidermal cells constitute approximately 40% of the total cell volume of the leaf blade indicating the great amount of leaf area that is epidermal cells. These results would seem to point out that the epidermis functions mainly as a barrier to penetration and not as an active site of accumulation since little radioactivity was found in that fraction, the epidermal cells, which occupied such a large percentage of the total leaf volume.

Influence of Gibberellins on Plant Processes

Gibberellins have the unique ability among plant hormones to stimulate extensive growth of intact plants. The stimulation of elongation growth has been found to involve one or more of the following three processes: increased cell division, stimulation of hydrolytic enzymes, or increases in wall plasticity (237). Andersen (5) reported that gibberellins mainly affect the frequency of mitosis of primary elongating stems. That means that a stem, which has been supplied with additional gibberellin, has more cells per internode than if it had been left with its own internal supply. Liu and Loy (133) found rates of cell proliferation that were about 2.5 times greater in gibberellic acid-treated seedlings of dwarf watermelon than in untreated seedlings, as determined by direct counts of pith cells in elongating hypocotyls. They attributed this to a shorter cell cycle time, with the resulting decrease being attributed to decreases in durations of the $G_1$ and $S$ periods. Sachs (195) indicated that cell
division was stimulated in the shoot apex, especially in the more basal meristematic cells which develop the long files of cortex and pith cells. Mitsan and Lang (173) indicated that gibberellic acid stimulated DNA synthesis in lentil epicotyls and Callebant, et al. (27) reported that gibberellic acid enhanced endomitotic DNA synthesis in pea epicotyls.

Zwar and Jacobson (238) found, working with aleurone, that after 16 h incubation, gibberellins stimulated the incorporation of uridine and adenosine into all species of RNA, but least into rRNA and tRNA and most into those species sedimenting between 5S and 10S, which is in agreement with what Key (119) reported. Rose and Crossman (192), working with wheat coleoptiles, found a long lag time in response to gibberellic acid compared to auxin. They reported that gibberellic acid had no stimulatory effect on RNA synthesis at 1 hour, but showed a stimulation of incorporation at 6 hours. Edwards (54) presented information indicating an enhancement of amino acid and protein synthesis in the cells of the meristem and developing tissues of the embryonic axis and cotyledons.

Gibberellins can stimulate cell growth through the stimulation of the hydrolysis of starch, fructosans, and sucrose into glucose and fructose molecules. These hexoses provide energy via respiration to contribute to cell wall formation, and also make the water potential of cells momentarily more negative. As a result of the decrease in water potential, water enters more rapidly and dilutes the sugars, causing cell expansion (52,171,196,223). Glassziou (63) found gibberellins promoted growth in sugarcane stems, resulting in part from an increase
in the synthesis of invertase which hydrolzed incoming sucrose. In dwarf pea, the activities of both invertase and amylase rise in parallel with growth (21).

Gibberellins sometimes increase wall plasticity (111). In oat internodes, in which growth promotion of young cells derived from the intercalary meristems is usually dramatic, there is no stimulation of cell division yet elongation due to gibberellins is 15 times greater than untreated sections (2). Jones and Kaufman (106), Kaufman (113,114) indicate that gibberelins caused a cessation of all cell division activity within intercalary meristems, yet markedly accelerated the rate of intercalary growth and cell lengthening. Haber and Luippold (66) investigated the gibberellin effects on cell expansion using the gamma plantlet system in which cell division had been eliminated in the seed by large doses of gamma irradiation. They found that gibberellins stimulated cell elongation in wheat plants developing without mitosis. They reported similar results from lettuce seeds (67). A similar phenomenon apparently explains gibberellin-promoted growth in lettuce hypocotyls (215).

Tort and Lagarde (219) examined the effects of gibberellic acid on mitosis in various zones of the apes of Stachys sieboldi and found that the more differentiated cell in an internode were stimulated to elongate while cells of the meristem medulla were stimulated to divide. Fisher (57) also found a stimulation of elongation and division in the intercalary meristem of decapitated culms of Cyperus alternifolius.

Kaufman, et al. (115) reported that gibberellins induced invertase
activity in *Avena* segments and that the amount of promotion closely paralleled the growth promotion caused by the range of gibberellin concentrations applied, meaning the greater the gibberellin the greater the promotion. They claimed that protein synthesis was necessary for gibberellin-promoted growth and invertase activity in *Avena* stem segments.

Wood and Paleg (232, 233) and Wood, et al. (234) indicated an interaction between gibberellins and membrane fluidity. They reported that gibberellins increased the permeability of the model membrane system, composed of various plant source lipids, sterols, and diacetyl phosphate. As a result of hormone treatment, the flux of uncharged molecules, such as glucose and sucrose, or charged ions, such as chromate, moved through the membrane at a greater rate.

Gibberellins can also overcome both dormancy by acting, in many species, as a substitute for low temperatures, long day, or red light (47, 88, 110, 196). In seeds, the principal gibberellin effect is to enhance cell elongation to push the radicle through the endosperm, seed coat, or fruit coat that restricts its growth. Buds have been less carefully studied. Whether or not a stimulation of cell division, in addition to elongation is necessary, is not known (196). Gibberellins are used to break dormancy and stimulate sprouting of potato tubers by dipping them into a 0.1 to 1.0 ug/ml solution of gibberellic acid prior to planting. This allows immediate planting of red cultivars which normally require 2 to 6 months of cold storage to break dormancy (185, 186).

Endosperm of half seeds of cereals treated with gibberellic acid
were found to respond to gibberellic acid in the same way that the entire seed responded to the hormone produced by the embryo. The procedure is as follows: first, by the rapid hydrolysis of starch to form reducing sugars, secondly, by hydrolysis of protein in the aleurone layer, third, by formation of amino acids, and fourth, by the appearance of inorganic phosphate (142). Work by Varner, et al. (221) led to the discovery that gibberellins controlled the synthesis of -amylase in the aleurone layer. Wareing and Phillips (223) described the process this way, the aleurone is a peripheral layer of protein-rich cells laying around the endosperm in seeds of grasses and cereals. During germination and early seedling growth the aleurone tissue is very active. The functions of the aleurone layer are to serve as a storage tissue prior to germination and as a source of a range of hydrolytic enzymes that are secreted to digest the reserves of the endosperm during germination. After imbibition of water by a non-dormant seed, the aleurone cells perform their function on receipt of appropriate hormonal signals. The principal hormone is gibberellin originating from the germinating embryo. A major effect of gibberellins on the aleurone cells is upon the enzyme -amylase. -amylase is not present in the dry, unimbibed barley seed, but appears in and is secreted from aleurone aler cells in response to gibberellins. Mayer and Poljakoff-Mayer (142) state that the -amylase formed in the aleurone arose by de novo synthesis of protein. Its synthesis was dependent on the synthesis of new mRNA and could be prevented by inhibitors of DNA dependent RNA synthesis.

Treatment of a)erone layers with gibberellins also enhances
the activity of a range of other enzymes, all of which are formed or activated in the aleurone cells but secreted to exert their hydrolytic action outside the protoplast of those cells. Some act in endosperm cells and others act on the walls of aleurone cells (223).

The use of gibberellins has been shown to inhibit tuber formation. Harada, et al. (74) reported that foliar sprays of 50 to 100 ppm gibberellic acid during the tuber formation of *Eleocharis kuroguwa* strongly inhibited the tuber formation of this paddy weed in rice, and consequently reduced the amount of weed in the following year. Foliar sprays of 50 to 100 ppm gibberellic acid applied to *Sagittaria trifolia* L. strongly inhibited tuber formation of this paddy weed in rice also (73).

Experiments have shown that the rate of cell division in the cambium can be regulated by levels of auxin and gibberellins. It has also been observed that the production and differentiation of xylem vessels can be favored by a relatively high ratio of auxin to gibberel­lin, where as phloem formation occurs when gibberellin level is high (4,223).

Gibberellins have been shown to increase height growth in many tree species (104,136,162,189). In general, however, gibberellin-induced increases in height growth are associated with decreases in the growth of leaves, branches, and roots, altered leaf, and a reduction in the root:top ratio. The influence of gibberellin application was ascribed to changes occurring in the normal dist­ribution of photosynthates. Little and Loach (132) reported that enhancement of height growth in balsam fir attained through treatment
with gibberellin could not be attributed to any gibberellin-induced increase in photosynthate production. Their results indicated that gibberellins acted by modifying the normal distribution of photosynthate, a disproportional amount being concentrated in the main stem.

Cole, et al. (32) found that gibberellins significantly increased forage yield of *Medicago sativa* by increasing stem production. Photosynthesis and respiration were reduced by gibberellic acid application. Roots and treated plants had lower percentages of free and acid hydrolyzed carbohydrates. The results indicated that gibberellic acid affected the "source to sink" relationship in carbohydrate translocation and storage in alfalfa, resulting in the photosynthate being metabolized in a different location in the plant, which caused an increase in the amount of stem tissue.

Moore and Ginoza (1168) showed that sugarcane stalk length, fresh weight, dry weight, and sucrose content, which are normally reduced when plants were grown under reduced sunlight and cool temperatures, could be increased with foliar applications of gibberellic acid.

Gibberellins have been shown to cause marked elongation of cucumber hypocotyls in the presence of the cotyledons, but promotion was only slight in their absence (112). The magnitude of the gibberellin response was directly related to the amount of cotyledon present. It was argued that a factor supplied by the cotyledons was necessary for the gibberellin growth response in this experiment.

Harada and Vergara (75) showed that gibberellin application increased the height of the plant and lengths of the internodes, leaf blades, and leaf sheaths but decreased tiller number in two dwarf
varieties of rice. Thus gibberellins are needed not only for stem
growth but also for leaf growth. It has also been reported that gib­
berellin exogenously applied to the roots of water hyacinth exert
significant effects on the pattern of development of clones (180,224).
A change in pattern of leaf morphologensis from a "float" to a
"canopy" form, as measured by length/width ratio of perioles, was
observed. As a consequence of this alteration in leaf morphology, the
plants became unstable and partially sank below the surface of the
water.

In several species of plants, gibberellin applications have
extended the juvenile period. This effect was first reported for ivy,
which showed a temporary reversion from mature to juvenile morphology
after gibberellin application (188). Salisbury and Ross (196) stated
that treating the leaf just above a lateral bud of a mature stem of
English Ivy induced juvenility in shoots that arose from that
particular lateral bud. Similar reports of enhancement of juvenility
have been reported for Citrus (33).

Henny (83), working with Dieffenbachia maculata "Perfection",
found that treating plants with gibberellic acid not only resulted
in flower production 4 to 5 months earlier than normal, but also
significantly increased the number of flowers per plant. Previous work
with other members of the Araceae family have shown stimulation of
flowering following treatment with gibberellins (76,145). Flowers
have been induced on conifer, which naturally take 10 to 20 years
to produce flowers, in 4 to 6 years by treating seedlings with
gibberellic acid sprays (223). Luukkanen and Johansson (137) found
that ethanolic sprays of gibberellins, applied to developing shoots of 12-year-old Scots pine grafts for two consecutive years during the shoot elongation period, resulted in increased flowering, with the effects being particularly distinct in male flowering. Read (185) also reported that gibberellins applied to gynoecious lines of cucumber promoted the formation of staminate flowers.

Many long-day plants and cold requiring biennials with rosetted growth have been induced to flower by applications of gibberellins. Lang (127,128) was the first to show that the long-day and cold-requiring biennial henbane (Hyoscyamus niger) could be induced to flower by applications of gibberellic acid to the apeces of plants grown under noninductive conditions. McComb (143) reported a similar type of response with Centaurium minus Moench.

In an extensive study with a number of plant species, Fletcher and Osborne (60,61) found that in most cases gibberellins were not effective in retarding leaf senescence whereas they clearly demonstrated that senescence of detached dandelion leaves could be delayed equally with either gibberellic acid or kinetin. Treatment of dandelion leaves with gibberellin delayed a decline in the levels of chlorophyll, RNA, and protein (58). Goldthwaite (65) reported that the net breakdown of chlorophyl in Rumex leaf disc was inhibited by the gibberellins GA₃ and GA₄⁺₇.

Other gibberellin-induced responses are parthenocarpic fruit induction in Prunus by spraying the flowers with a solution of gibberellins (223) and the induction of negative geotropic responses in Yellow nutsedge, strawberry clover, Circea intermedia, and red
clover (18,19,48,210).

Effects of Gibberellins on Translocation

There are reports that gibberellin applications increase the basipetal flow of IAA in plant tissue. Jacobs and Case (99) found that gibberellin added with auxin to decapitated shoots of Pisum sativum significantly increased the duration of inhibition of side shoots. The presence of gibberellic acid in the apical paste caused much more 14C-IAA to be extracted farther down the stem after 2 days, but by 4 days this effect of gibberellic acid was no longer apparent. Pilet (182) observed that gibberellic acid increased the uptake and movement of 14C-IAA in isolated segments of Lens culinaris stems.

Gibberellic acid has been reported to either increase export of photosynthates from leaves (37,84) or to decrease them (69). Halevy, et al. (69) reported that gibberellic acid not only decreased export of photosynthates, but also enhanced their acropetal movement. This has been supported by other workers (37,79,102,202), while some have presented evidence for an enhanced basipetal movement (84,225).

Mulligan and Patrick (170) found that when gibberellie acid was applied as a dispersion in aqueous lanolin to the stumps of decapitated stems of Phaseolus vulgaris plants a promotion in the transfer of 14C and 32P labelled assimilates to the site of hormone application occurred. Measurements of the component transfer process, operating between source and sink (site of hormone application), showed that gibberellic acid was not acting to promote assimilate transfer by increasing photosynthetic rate of, or the assimilate export rate from the source, nor by altering the mobilizing ability of the
competing root sink. This enhanced transport took place in the absence of any changes in the net photosynthetic rate (source activity) or relative export rate (sieve tube loading) from the primary leaves. This indicates that the gibberellic acid applied to the cut surface of decapitated, mature stem tissue stimulated acropetal assimilate movement by acting locally at the point of application. Umoessien and Forward (220) wrote that gibberellic acid altered the distribution of the products of assimilation of $^{14}$CO$_2$ from a sunflower leaf, but not immediately or directly. The leaf that received the carbon label normally exported sugar mainly basipetally. The pattern was not consistently changed by gibberellic acid during the first 2 hours when the leaf was rapidly exporting labelled photosynthate. Therefore the major effect of the gibberellin was not on the initial direction of movement from the source. It was, rather, on the redistribution of $^{14}$C that occurred after the plant leaf had almost ceased exporting photosynthate labelled by the pulse of $^{14}$CO$_2$, i.e. between 2 to 6 hours. The added gibberellic acid enhanced the accumulation of $^{14}$C in the apical segments of the plant, especially the shoot at the expense of lower portions of the plant, especially the roots. Whenever it was applied, the major effect of gibberellic acid was on transfer of sucrose from leaf to root via the phloem, its metabolism in the root, and re-export to the shoot of some part of its carbon in some form of organic acids or amino compounds, in the xylem sap. This indicates that the role of gibberellic acid was to enhance the re-export phase. They found that gibberellic acid-stimulated accumulation was active, thus suggesting an attraction to an
activated sink rather than a general increase in transport of labelled materials.

Marea, et al. (1410 discovered that the effects and consequences of the application of gibberellic acid to potato tubers increased the "sink" strength of the stolen, non-growing tubers, and the basal tissue of the tuber. This possibly via an enhancement or facilitation of phloem unloading which decreased the overall "sink" strength of the tubers. Thomas, et al. (218) stated that gibberellic acid increased the shoot to storage ratio and decreased root yield by up to 35% in carrots.

Harris, et al. (79) presented evidence indicating that the application of gibberellic acid to carnations diverts assimilates to the flower and hastens development toward anthesis. It was later found that application of IAA or gibberellic acid to the stump would partially restore the pattern back to the regular developmental pattern.

Halevy and Wittmer (70) reported that gibberellic acid increased Rb uptake but did not affect total translocation from the treated leaf. Translocation was directed to the upper vegetative parts and markedly reduced to the roots.

**Gibberellins and Herbicide Interaction**

The chloroacetamide herbicides are widely used for the control of grasses and broadleaf weeds in corn and soybeans. Depending upon the weed species examined, early weed seedling development has been shown to be inhibited through root, shoot, or a combination of shoot and seed exposures (3,123). When applied pre-emergence, these chemicals apparently inhibit seed germination or development of germinating seedlings (72), indicating a basic inhibitory effect on
one or more metabolic processes upon which seed germination and early seedling growth are dependent (184). It has been reported that alachlor and propachlor (2-chloro-N-isopropylacetanilide) inhibit gibberellic acid-induced α-amylase production in de-embryonated barley seed, which suggests that this effect could be related to an effect on protein synthesis (41). Chang, et al. (30) found that alachlor inhibited growth oat seedling and that pre-treatment with gibberellic acid overcame this inhibition. Roa and Duke (184) stated that alachlor, propachlor, and pyrochlor (2-chloro-N-(1-methyl-2-propynyl)acetanilide) inhibited gibberellic acid induced production of protease and α-amylase, with the protease being the most sensitive of the two. The degree of inhibition of protease and α-amylase production caused by alachlor was equivalent to that caused by cyclohexamide, puromycin, and actinomycin-D, known protein and nucleic acid synthesis inhibitors. It was shown further that higher gibberellic acid concentrations reversed the inhibition of protease and α-amylase synthesis caused by alachlor in de-embryonated seed but did not reduce the effect of alachlor on barley seed germination and growth. This would indicate that the alachlor effect on germination and and seedling growth involves more than an inhibition of protease and α-amylase synthesis. Warsaiah and Harvey (172) found that alachlor significantly inhibited growth of epicotyl segments while gibberellic acid usually increased growth. Biggerellic acid in combination with alachlor caused significant increase in growth compared to alachlor alone, resulting in growth comparable to that occurring in untreated tissues.

Wilkinson (229) found that alachlor decreased 14-C incorporation
of [2-\textsuperscript{14}C] mevalonic acid into [14\textsuperscript{c}-C] geraniol (geranyl pyrophosphate), [14\textsuperscript{c}-C] kaurenol, and [13\textsuperscript{c}-C] squalene and increased 14\textsuperscript{c}-C incorporation into 14\textsuperscript{c}-C geranylgeranyl pyrophosphate and [14\textsuperscript{c}-C] kaurene (see Fig. 2). Since farnesol is a precursor to the sterols and triterpenes, decreased 14\textsuperscript{c}-C incorporation into the sterols and triterpenes should have resulted in increased concentration of geranylgeranyl pyrophosphate \( \rightarrow \) kaurene \( \rightarrow \) kaurenol. Yet 14\textsuperscript{c}-C incorporation into [14\textsuperscript{c}-C] kaurenol was decreased. Thus, alachlor inhibited the kaurene to kaurenol conversion, which resulted in decreased gibberellic acid synthesis. It was also found that alachlor inhibited conversion of farnesol (farnesyl pyrophosphate) to sterol and that these inhibitions were partially reversed by the addition of a safener.

Wilkinson (227) reported that carotene content in sorghum leaves was decreased by metolachlor. Thus, terpenoid biosynthesis was influenced by metolachlor. Gibberellins are terminal products of plant terpenoid biosynthesis, and gibberellic acid reverses metolachlor inhibition of growth at specific ratios of gibberellic acid and metolachlor but not at other ratios. Thus, one effect of metolachlor on plants may be an inhibition of gibberellic acid synthesis which results in shoot and root growth reductions as metolachlor concentration increases. In later work Wilkinson (228) found that 1\textsuperscript{c}-C incorporation of 2-1\textsuperscript{c}-C mevalonic acid into kaurene was inhibited by \( 10^{-7} \) to \( 10^{-4} \) M metolachlor. [14\textsuperscript{c}-C] geranylgeranyl pyrophosphate content increased, 14\textsuperscript{c}-C farnesyl pyrophosphate content was not altered, and 14\textsuperscript{c}-C geranyl pyrophosphate content decreased. Also the total 14\textsuperscript{c}-C incorporation was decreased by metolachlor. The use of a
safener was found to reverse the inhibition of kaurene synthesis and since the biosynthetic processes are mevalonic acid → geranyl pyrophosphate → farnesyl pyrophosphate → geranylgeranyl pyrophosphate → copalyl pyrophosphate → kaurene, these data corroborate a proposed inhibition of gibberellic acid biosynthesis between geranylgeranyl pyrophosphate and kaurene as well as a partial blockage between mevalonic acid and geranyl pyrophosphate.

Donald, et al. (46) found that hydroponic root treatment with gibberellic acid prevented the leaf stunting previously seen due to EPTC application and that most abnormalities in foliar display and height from treatment with μM EPTC were prevented by increasing gibberellic acid levels from 0.5 to 10 μM during the course of a week. Wilkinson and Ashley (231) found that gibberellic acid content of wheat, as measures by gas-liquid chromatography, was decreased 96.4% by 125.0 ppbw EPTC, which would demonstrate the effect of EPTC on the biosynthetic pathway of gibberellic acid. When $^{14}$C mevalonic acid was added to the nutrient solution, incorporation into total kaurenoic acid was decreased 90% by 250 ppbw EPTC. Metabolism of kaurene was reduced by 250 ppbw EPTC, with the resultant 3-fold accumulation of kaurene. Wilkinson later reported (230) that the inhibition of gibberellic acid precursor biosynthesis by EPTC occurred primarily at the cyclization of geranylgeranyl pyrophosphate, which occurs before kaurene is synthesized. This inhibition was reported to be reversed by the safener R-25788 (N,N-diallyl-2,2-dichloroacetamide).

Leavitt and Penner (129), studying potential antidotes against acetanilide herbicide injury to corn, concluded that gibberellic acid
increased corn growth sufficiently to overcome the stunting caused by EPTC and metolachlor as compared to the ethanol control, but it did not prevent herbicide injury symptoms such as leaf stunting, leaf rolling and twisting, and stem swelling.

A number of reports have indicated that exogenous applications of gibberellic acid increased the uptake and movement of herbicides. Asgton (7,8) studying the effects of gibberellic acid on the absorption, translocation, and degradation of 2,4-D in red kidney bean plants, concluded that the increased amount of 2,4-D in the reservoir of the gibberellic acid treated plant after 24 hours resulted from 2,4-D being translocated at a relatively higher rate in gibberellic acid treated plants and not due to increased absorption. Pieterse and Rooda (181) reported that gibberellic acid markedly enhanced the effect of 2,4-D on water hyacinth (Eichornia crassipes) by reducing the amount of 2,4-D required for control from 1000 g/ha to 100 g/ha. From studies of the effects of growth regulators on 2,4,5-T translocation in bean plants, Basler (14,15) reported that gibberellic acid appeared to enhance the acropetal translocation of 2,4,5-T and inhibited basipetal translocation. This resulted in increased accumulation of 2,4,5-T in the young shoots, with the effect being more pronounced 24 hours after treatment. Gibberellic acid appeared to have inhibited the basipetal movement of 2,4,5-T to root and nutrient solution (14).

Devlin (40) demonstrated that treatment of redtop (Agrostis gigantea Roth) with gibberellic acid increased its sensitivity to simazine (2-chloro-4,6-bis(ethylamino)-s-triazine). In addition,
Devlin, et al. (42) found that both IAA and gibberellic acid enhanced the herbicidal effect of silvex (2-(2,4,5-trichlorophenoxy)propionic acid) on poison ivy. In that study, residue analysis showed more silvex in those plants which were treated with IAA or gibberellic acid than those plants which received no plant growth regulator. Devlin and Yaklich (44) found that pre-treatment of bean plants resulted in those plants absorbing and accumulating more naptalam at all concentrations of gibberellic acid from $2.9 \times 10^{-6}$ to $8.6 \times 10^{-4}$ M than those which were not treated with the gibberellin. When the different parts of the plant were analyzed, separately, for naptalam, the leaf area was found to have been influenced most by gibberellic acid applications. In addition, Devlin and Karczmarczyk (43) got results which indicated that simultaneous applications of gibberellic acid and naptalam resulted in wheat seedlings being stimulated in their uptake of naptalam.

Gibberellin-enhanced response of bean plants and Canada thistle to bentazon and glyphosate (209) showed that the enhanced phytotoxicity may be due to changes in acropetal translocation and accumulation in leaf sinks. They also noted that the leaf area of gibberellin-treated Canada thistle was significantly greater than that of non-gibberellin-treated plants. Thus, enhanced phytotoxicity caused by gibberellin could be the result of delayed leaf maturity, thereby causing more active metabolic sinks for herbicide accumulation.

**Fluazifop-butyl**

Fluazifop-butyl (Figure 3) was introduced by Isihara Sangyo Kaisha Ltd in 1980 and is being jointly developed with I.C.I. Ltd as a
Figure 3. Chemical structure of fluazifop-butyl.
post-emergence herbicide for the selective control of annual and perennial grass weeds, primarily for use in dicotyledonous crops. Extensive field trials have shown that it can be used in over sixty different crops to control the major grass weeds (59,96). Fluazifop-butyl is quickly absorbed through leaf surfaces. Plowman, et al, (183) reported that simulated rain (28 mm) applied 1, 6, and 24 hours after spraying showed that even after 1 hour, rainwashing has little effect on fluazifop-butyl activity.

The work by Rosser, et al. (193) on rhizome johnsongrass demonstrated that $^{14}$C labelled fluazifop-butyl translocated throughout the plant but accumulated primarily in meristematic regions, where it is reported to interfere with ATP production (96). Areas of accumulation were young johnsongrass tillers, meristematic areas above the treated leaf, rhizome, and secondary shoots from rhizomes connected to the treated plant.

It has been reported that fluazifop-butyl controls perennial weeds at rate of 0.125 to 0.6 kg/ha for rhizome johnsongrass in soybeans and at rates as low as 0.06 kg/ha for quackgrass (Agropyron repens (L.) Beauv.) in alfalfa (50,51,81,187). Buhler and Burnside (22) reported excellent annual weed control both pre-emergence and post-emergence in soybeans, but found less herbicide action when applied pre-emergence. Similar results were obtained for annual grass weed control in soybeans and blueberries, with post-emergence applications of fluazifop-butyl (80,206,222).

Smith (205) found no phytotoxicity to juniper, cotton Easter, euonymus, English ivy, pachysandra, myrtle, and purple winter
creeper at rates of 0.5 to 1.0 kg/ha fluazifop-butyl.

Sarpe and Dinu (198) showed it was safe to apply fluazifop-butyl at a crop height of 10 to 20 cm and at the flowering stage of soybeans even at rates of 3.0 kg/ha. Buhler and Burnside (23) reported on the influence of spray volume on the phytotoxicity of fluazifop-butyl at various concentrations. They found that at the lower rate (0.05 kg/ha) fluazifop-butyl demonstrated more effective control when applied in a carrier volume of 48 L/ha rather than at greater volumes. They also found that 0.15 kg/ha was effective at a greater carrier volume (190 L/ha) and that greater herbicide concentrations were not greatly influenced by carrier volume.

Fluazifop-butyl has been shown to readily mix with tung oil (38). In this experiment soybean seeds were coated with a mixture of fluazifop-butyl and tung oil, which polymerized to form a thin, hard coat around the soybeans. The results indicated that fluazifop-butyl did not cause a reduction in number of soybean plants emerging but it did result in a reduction of *Eleusine indica* (test plant) plant weights when compared to the untreated control plants.
GIBBERELIC ACID ENHANCED PHYTOTOXIC RESPONSE
OF JOHNSONGRASS (SORGHUM HALPENSE) TO
POST-EMERGENCE GRAMINICIDES

Introduction

The first reported case of enhanced herbicidal activity by gibberelic acid involved red kidney bean (*Phaseolus vulgaris* L.) plants treated with gibberelic acid 48 h prior to application of 2,4-D (2,4-dichlorophenoxyacetic acid) and demonstrated that gibberelic acid applications could alter the translocation pattern of the applied herbicide (1,2). Similar results were obtained using bean plants treated with 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) where gibberelic acid increased accumulation of 2,4,5-T in the young shoots and decreased accumulation in the roots and nutrient solution (3,4).

Other reports have indicated that application of gibberelic acid to plants, either as a pre-treatment or in combination with the herbicide, enhanced the physiological response to the herbicide. Pieterse and Rooda (18) found that gibberelic acid markedly enhanced the effect of 2,4-D on water-hyacinth (*Eichhornia crassipes* (Mart.) Solms-Laubach) when applied in combination and Devlin (9) demonstrated that treatment of redtop (*Agrostis alba* L.) with gibberelic acid increased its sensitivity to simazine (2-chloro-4,6(ethy lamino)-s-triazine). It has also been found that the phytotoxicity of silvex
(2-(2,4,5-trichlorophenoxy)propionic acid) on poison ivy (Rhus toxicodendron L.) can be enhanced through applications of gibberellic acid added in combination with the herbicide (10). These findings would suggest that applications of this growth regulator are stimulating uptake and/or translocation of the applied herbicide which is increasing the plants susceptibility to the chemical.

Studies with naptalam (N-l-napththylphthalamic acid) have shown that gibberellic acid stimulated naptalam uptake by wheat (Triticum vulgare L.) seedlings from the nutrient solution (11). Similarly, pre-treatment of bean plants (Ph. vulgaris L. var. 'Black Valentine') with gibberellic acid was reported to result in greater absorption and accumulation of naptalam and at its optimal concentration, gibberellic acid pre-treated plants took up 58% more naptalam than untreated plants (13).

More recently, Sterrett and Hodgson (22) reported that gibberellic acid pre-treatment of bean plants in the growth chamber more than doubled the herbicidal effect of bentazon (3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide) while pre-treatment of Canada thistle (Cirsium arvense (L.) Scop.) increased the herbicidal effect of bentazon and glyphosate (N-(phosphonomethyl) glycine) four-and two-fold, respectively.

Johnsongrass (Sorghum halepense (L.) Pers.) is the principle weed of cotton and sugarcane as well as soybeans, sorghum, and orchards in the continental United State and Hawaii (15). In 9 of the 11 Southern states, johnsongrass ranks among the ten most costly weeds irrespective of crop (17). It was the aim of this research to determine
if gibberellic acid could enhance herbicidal response, in johnsongrass, of several of the new post-emergence graminicides. Based on what was observed, a more elaborate evaluating system was developed and used to monitor phytotoxic responses seen in a growth chamber environment. Gibberellic acid enhanced the phytotoxicity of fluazifop-butyl at 0.1 or 0.2 kg/ha but not at other rates nor did gibberellic acid enhance the other herbicides evaluated.

Materials and Methods

Field Experiments. Field experiments were conducted during the summers of 1981 and 1982 at research plots located near Fox, Ohio. An area of heavy johnsongrass infestation was plowed in the fall and prepared in the spring prior to initiation of each experiment. The 3 by 7 meter plots were arranged in a randomized complete block experimental design with four replications.

Sethoxydim, fluazifop-butyl, haloxyfop-methyl, CGA 82725, and Ro 13-8895 were applied in single applications at rates ranging from 0.1 to 0.4 kg/ha. Sethoxydim was also applied in split applications at 0.2 + 0.2 and 0.3 + 0.3 kg/ha. Gibberellic acid was tank mixed at 2.0, 3.0, and 7.0 g ai/ha with the above herbicide rates to an additional set of plots. A non-ionic surfactant, X-77 (alkylaty-polyoxyethylene glycol), was added to the treatment solution at 1% v/v for sethoxydim and CGA 82725 and 0.1% for fluazifop-butyl and Ro 13-8895 in 1981 while a crop oil concentrate (a blend of paraffin base petroleum (83%) and polyol fatty acid esters and polyethoxylated derivatives (15%)) was added at 1% v/v to all treatments in 1982. Applications were made with a CO₂ powered backpack sprayer delivering
280 L/ha at 172 kPa.

A tank mix of metribuzin (4-amino-6-tert-butyl-3-(methylthio)-as-triazine-5(4H)one) and chloramben (3-amino-2,5-dichlorobenzoic acid) at 0.28 kg ai/ha and 2.25 kg ai/ha, respectively, were applied pre-emergence for broadleaf weed control June 16, 1981 and May 27, 1982. On July 10, 1981 a post-emergence application of pre-mixed naphtalam plus dinoseb (N-l-naphthylphthalamic acid plus 2-sec-butyl-4,6-dinitrophenol) and 2,4-DB (2,4-dichlorophenoxy)butyric acid) at rate of 1.1 kg ai/ha and 0.14 kg ai/ha, respectively, were applied with a tractor-mounted sprayer.

The phytotoxic response on johnsongrass was rated at weekly intervals, beginning two weeks following the initial application. A rating scale of 1 (no visual phytotoxic response) to 10 (complete death to the entire population) was used. Rating continued for six weeks in 1981 and for nine weeks in 1982. Each set of weekly phytotoxicity ratings were subjected to analysis of variance, with the means being separated by Duncan's multiple range test.

Growth Chamber Experiments. During the fall of 1982, a study was conducted in a controlled environment chamber incorporating results of the field experiments. The temperatures and photoperiods were set for 32 C and 16 h days and 24 C, 8 h nights. A light intensity of 30 klx was obtained with fluorescent and incandescent lamps. The relative humidity fluctuated between 60 and 70%. Three-node johnsongrass rhizomes pieces were planted in 2-L pots filled with a mixture of soil:sand:peat moss (2:1:1 v/v/v). Twenty-two days following planting, uniform johnsongrass plants were treated with fluazifop-
butyl, the only herbicide tested in the field which demonstrated a gibberellic acid response, at zero, 0.1, 0.2, and 0.4 kg/ha in combination with gibberellic acid at zero, 3.0, and 7.0 g/ha. A crop oil concentrate was added, at a 1% v/v ratio, to each treatment solution. The entire plant was sprayed using a hand-held atomizer at a rate of 5 ml per plant. Each leaf of each plant was monitored daily for phytotoxic symptoms. These visual observations were given a numerical value based on the rating scale developed for this experiment (Table 1). The data on each leaf were subjected to analysis of variance. The mean separation was done using Fisher's LSD procedure to facilitate graphic representation. There were four replications per experiment which was repeated three times.

Results and Discussion

Field Experiments. Weekly evaluations of several new post-emergence herbicides applied to johnsongrass, alone or in combination with gibberellic acid, indicated that of the graminicides screened only with fluazifop-butyl was there an increased phytotoxicity due to the addition of gibberellic acid (Tables 2,3). The phytotoxicity ratings, which began 14 days after treatment, showed that 2 g/ha gibberellic acid was sufficient to alter plant response to 0.1 kg/ha fluazifop-butyl in three of the six evaluation periods during the first year (Table 2). Gibberellic acid did not enhance phytotoxicity of 0.2 kg/ha fluazifop-butyl during this first year.

In the second year the addition of gibberellic acid at the 3.0 and 7.0 g/ha rates again altered plant response to fluazifop-butyl, but at different herbicide rates (Table 3). At 0.1 kg/ha fluazifop-
Table 1. The rating scale used in the visual evaluation of leaves of johnsongrass treated with fluazifop-butyl, gibberellic acid, or combinations of the two.

<table>
<thead>
<tr>
<th>Rating</th>
<th>General Description</th>
<th>Detailed Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No effect</td>
<td>Leaf appeared healthy</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Slight pale green discoloration</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Chlorotic speckling seen, yet not widespread on the leaf</td>
</tr>
<tr>
<td>4</td>
<td>Slight effect</td>
<td>Necrotic speckling associated with chlorotic blotches, which are not widespread on the leaf</td>
</tr>
<tr>
<td>5</td>
<td>Moderate effect</td>
<td>Slight interveinal chlorosis over majority of leaf</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>Moderate interveinal chlorosis over majority of leaf</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>Heavy chlorosis over majority of leaf with slight necrosis and slight curling</td>
</tr>
<tr>
<td>8</td>
<td>Severe effect</td>
<td>Heavy chlorosis and moderate necrosis associated with moderate leaf margin curl</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>Heavy chlorosis and heavy necrosis covering the leaf, extensive leaf margin and tip curling</td>
</tr>
<tr>
<td>10</td>
<td>Terminal effect</td>
<td>Leaf dried, curled, and brittle</td>
</tr>
</tbody>
</table>
Table 2. Phytotoxic response of rhizome johnsongrass treated with selected herbicides with and without exogenously applied gibberellic acid (1981).

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>Herbicide</th>
<th>Rate (kg/ha)</th>
<th>GA3 (g/ha)</th>
<th>Days after treatment application when evaluated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td>CGA 82725</td>
<td>0.2</td>
<td>0</td>
<td>9.4a</td>
<td>9.6abc</td>
</tr>
<tr>
<td>CGA 82725</td>
<td>0.2</td>
<td>2</td>
<td>9.4a</td>
<td>9.7ab</td>
</tr>
<tr>
<td>CGA 82725</td>
<td>0.4</td>
<td>0</td>
<td>9.1ab</td>
<td>9.4abc</td>
</tr>
<tr>
<td>CGA 82725</td>
<td>0.4</td>
<td>2</td>
<td>9.4a</td>
<td>9.9a</td>
</tr>
<tr>
<td>Flusazifop-butyl</td>
<td>0.1</td>
<td>0</td>
<td>4.5e</td>
<td>5.9e</td>
</tr>
<tr>
<td>Flusazifop-butyl</td>
<td>0.1</td>
<td>2</td>
<td>7.4cd</td>
<td>8.1abed</td>
</tr>
<tr>
<td>Flusazifop-butyl</td>
<td>0.2</td>
<td>0</td>
<td>7.8bc</td>
<td>8.4abcd</td>
</tr>
<tr>
<td>Flusazifop-butyl</td>
<td>0.2</td>
<td>2</td>
<td>7.9bc</td>
<td>8.1abed</td>
</tr>
<tr>
<td>Ro 13-8895</td>
<td>0.1</td>
<td>0</td>
<td>8.9ab</td>
<td>9.4ab</td>
</tr>
<tr>
<td>Ro 13-8895</td>
<td>0.1</td>
<td>2</td>
<td>9.4a</td>
<td>9.4abc</td>
</tr>
<tr>
<td>Ro 13-8895</td>
<td>0.2</td>
<td>0</td>
<td>9.6a</td>
<td>9.9a</td>
</tr>
<tr>
<td>Ro 13-8895</td>
<td>0.2</td>
<td>2</td>
<td>9.9a</td>
<td>9.9a</td>
</tr>
<tr>
<td>Sethoxydim</td>
<td>0.2</td>
<td>0</td>
<td>6.1d</td>
<td>7.0d</td>
</tr>
<tr>
<td>Sethoxydim</td>
<td>0.2</td>
<td>2</td>
<td>7.4cd</td>
<td>7.5cd</td>
</tr>
<tr>
<td>Sethoxydim</td>
<td>0.3</td>
<td>0</td>
<td>7.4cd</td>
<td>7.3cd</td>
</tr>
<tr>
<td>Sethoxydim</td>
<td>0.3</td>
<td>2</td>
<td>8.4abc</td>
<td>7.5bcd</td>
</tr>
<tr>
<td>None (check)</td>
<td>...</td>
<td>...</td>
<td>1.0f</td>
<td>1.0f</td>
</tr>
</tbody>
</table>

aAll treatments had a non-ionic surfactant added.

bGA3 is the gibberelin used in this study.

cThe phytotoxic response rating scale consisted of going from 1, a healthy stand of johnsongrass, to 10, the stand being completely dried curled and dead.

dRating values, in the same column, followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.
Table 3. Phytotoxic response of rhizome johnsongrass treated with selected herbicides with and without exogenously applied gibberellic acid (1982).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after treatment application when evaluated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbicide</td>
<td>Rate (kg/ha)</td>
</tr>
<tr>
<td>CQA 82725</td>
<td>0.2</td>
</tr>
<tr>
<td>CQA 82725</td>
<td>0.2</td>
</tr>
<tr>
<td>CQA 82725</td>
<td>0.2</td>
</tr>
<tr>
<td>CQA 82725</td>
<td>0.4</td>
</tr>
<tr>
<td>CQA 82725</td>
<td>0.4</td>
</tr>
<tr>
<td>Haloxyfop-butyl</td>
<td>0.1</td>
</tr>
<tr>
<td>Haloxyfop-butyl</td>
<td>0.1</td>
</tr>
<tr>
<td>Haloxyfop-butyl</td>
<td>0.1</td>
</tr>
<tr>
<td>Haloxyfop-butyl</td>
<td>0.2</td>
</tr>
<tr>
<td>Haloxyfop-butyl</td>
<td>0.2</td>
</tr>
<tr>
<td>Haloxyfop-butyl</td>
<td>0.2</td>
</tr>
<tr>
<td>Haloxyfop-methyl</td>
<td>0.15</td>
</tr>
<tr>
<td>Haloxyfop-methyl</td>
<td>0.15</td>
</tr>
<tr>
<td>Haloxyfop-methyl</td>
<td>0.15</td>
</tr>
<tr>
<td>Haloxyfop-methyl</td>
<td>0.3</td>
</tr>
<tr>
<td>Haloxyfop-methyl</td>
<td>0.3</td>
</tr>
<tr>
<td>Haloxyfop-methyl</td>
<td>0.3</td>
</tr>
<tr>
<td>Sethoxydim, Sethoxydim</td>
<td>0.2 + 0.2</td>
</tr>
<tr>
<td>Sethoxydim, Sethoxydim</td>
<td>0.2 + 0.2</td>
</tr>
<tr>
<td>Sethoxydim, Sethoxydim</td>
<td>0.2 + 0.2</td>
</tr>
<tr>
<td>Sethoxydim, Sethoxydim</td>
<td>0.3 + 0.3</td>
</tr>
<tr>
<td>Sethoxydim, Sethoxydim</td>
<td>0.3 + 0.3</td>
</tr>
<tr>
<td>Sethoxydim, Sethoxydim</td>
<td>0.3 + 0.3</td>
</tr>
<tr>
<td>None (Check)</td>
<td>...</td>
</tr>
</tbody>
</table>

<sup>a</sup>All treatments had a crop oil concentrate added.

<sup>b</sup>GA<sub>3</sub> is the gibberellin used in this study.

<sup>c</sup>The phytotoxicity rating scale goes from 1, a healthy stand of johnsongrass, to 10, the stand of johnsongrass being completely dried, curled, and dead.

<sup>d</sup>Rating values, in the same column, followed by the same letter are not different at the 0.05 level, according to Duncan's multiple range test.
butyl, the addition of 3.0 g/ha of gibberellic acid decreased phytotoxicity 35 days after treatment, which was maintained for the rest of the ten-week evaluation period, while the addition of 7.0 g/ha showed no visible effect. However, the addition of either 3.0 or 7.0 g/ha of gibberellic acid did enhance the phytotoxicity of 0.2 kg/ha of fluazifop-butyl. The 3.0 g/ha rate of gibberellic acid was not as effective as the 7.0 g/ha rate, which increased the phytotoxicity of fluazifop-butyl on eight of the nine evaluation dates.

Growth Chamber Experiments. Based on the previous developed rating scale (Table 1), comparisons of phytotoxicity in an older leaf with the youngest fully expanded leaf indicated differential responses with regards to herbicide rate, leaf age, and gibberellic acid concentration (Figure 1). The older leaf was the third leaf from the bottom of the plant. In the absence of fluazifop-butyl, there was no great differences in the older leaf in response to the different concentrations of gibberellic acid. In the younger leaf there was also no response to gibberellic acid until 12 days after application when the 7.0 g/ha rate yielded a slight phytotoxic response. It was apparent that the older leaf was in the process of natural senescence whereas the younger leaf was still active.

In the older leaf there were no responses at the 0.1, 0.2, and 0.4 kg/ha to additions of gibberellic acid. Neither was there response in the younger leaf to 0.1 and 0.4 kg/ha fluazifop-butyl when gibberellic acid was added. The younger leaf of the plants treated with 0.2 kg/ha fluazifop-butyl showed a response different from the older leaf. Here both rates of gibberellic acid resulted in increased phytotoxicity
Figure 4. The visual response to fluazifop-butyl at specified rates, with different levels of gibberellic acid, as influenced by leaf age.
of fluazifop-butyl to the johnsongrass. The 7.0 g/ha concentration demonstrated an accelerated response very rapidly and for about four days the response was almost linear.

Excluding those plants which received only gibberellic acid, it was found, when comparing the age of the leaf, that the older leaf responded to the herbicide, alone or in combination with gibberellic acid, with a more decreased rate of response than did the younger leaf.

The findings of the field screening experiment demonstrated that the use of gibberellins enhanced only one of the graminicides evaluated, fluazifop-butyl, and that phytotoxicity was found to differ from year to year. The effects of the application of gibberellic acid with fluazifop-butyl, as seen in the field, could be the result of increased translocation. Ashton (1) stated that gibberellic acid does not appear to affect the absorption of 2,4-D and that the increased effect is related to increased translocation which becomes apparent three to five days after application of the herbicide. The effects of gibberellic acid on the partitioning of photosynthates indicates that photosynthetic partitioning is altered with an increased movement to the apical parts of the plants (16,21). This response is in harmony with what has been reported for the influence of gibberellic acid on selected herbicides (3,4,22). The greater acropetal translocation would result in greater accumulation of the fluazifop-butyl toxicant in the apical growing points of johnsongrass. This would result in a more pronounced visual phytotoxic response, as was found.

Comparing the phytotoxic influence of gibberellic acid with johnsongrass for fluazifop-butyl on a day by day and leaf by leaf
basis indicated that an enhanced phytotoxic response was seen at the 0.2 kg/ha rate only with the younger leaf and not with the older leaf. The difference in response due to leaf age is consistent with what is expected regarding translocation of photosynthates and leaf age. It is understood that younger leaves are greater exporters than are older leaves, with the younger leaves exporting to the apical portion of the plant and reaching a maximum rate of export sometime after the leaf is 70% unfolded (5,6,7,8). An older leaf, one past maturity along with a thicker cuticle which would also act as an obstacle, would be expected to have a much reduced rate of translocation. This would result in a lesser amount of the toxicant reaching the site of action. The influence of the gibberellic acid as seen with the younger leaf of the 0.2 kg/ha fluazifop-butyl treated plant would lend support to the idea of increased acropetal translocation. Remembering that the entire plant was treated, if the addition of gibberellic acid is causing an increased acropetal translocation to the more active metabolic sinks as has been reported (23) then there would be greater movement of the toxicant to the apical portions of the plant. This would be seen as the greater visual phytotoxic response.

In influence of gibberellic acid on the maturation or senescence of leaves has been reported indicating that gibberellic acid retards the senescence process in certain plant species (12,20,24). Sterrett and Hodgson (22) noted that the leaf area of gibberellic acid-treated Canada thistle was greater than that of non-gibberellic acid-treated plants, suggesting that the delay in maturation would cause more active metabolic sinks to persist.
With fluazifop-butyl the suggested site of action is in the meristematic tissue of the plant (19). Alterations in the meristematic regions of the plant would disrupt the translocation mechanism of a leaf and the leaf would be forced into a state of senescence. The gibberellic acid in the spray solution could be delaying the net breakdown of chlorophyll in the leaf as has been reported for Rumex leaves (14). This would be seen as a delay in the overall visual death of the leaf, as was indicated with the leaf monitoring experiment.

The addition of gibberellic acid to spray mixtures was found to result in a greater phytotoxicity to fluazifop-butyl, with this increase being attributed, but not substantiated, to the acropetal translocation of the herbicide or in the delaying of the senescence process in the gibberellic acid treated plant leaves,
Literature Cited


GIBBERELLIC ACID ENHANCEMENT OF FLUAZIFOP-BUTYL ABSORPTION AND PARTITIONING IN JOHNSONGRASS (SORGHUM HALEPENSE)

Introduction

One of the physiological responses to gibberellins has been its influence on the partitioning of photosynthates. Gibberellins have been reported to increase export of photosynthates from leaves (7,11) but also to decrease export (9). Of the photosynthates which are exported several reports have indicated there is enhanced acropetal movement (7,8,9,10,13,18,20) while other reported enhanced basipetal movement (11,12) as a result of gibberellin application. Mulligan and Patrick (13) indicated that in Phaseolus vulgaris gibberellic acid was not acting to promote assimilate transfer by increasing the photosynthetic rate, nor by increasing assimilate export rate from the source, neither by altering the mobilizing ability of the competing root sink. Enhanced transport took place in the absence of any change in the net rate of photosynthesis. This was supported by Umoessien and Forward (20) who concluded that the stimulated accumulation in the shoot resulted from attraction to an activated sink rather that a general increase in transport of metabolities.

The influence of gibberellic acid on herbicidal partitioning was first reported with 2,4-D (2,4-dichlorophenoxyacetic acid) and indicated that response to gibberellic acid was the result of increased trans-
Studies of gibberellic acid and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) demonstrated that gibberellic acid appeared to enhance the acropetal translocation of 2,4,5-T and inhibited basipetal translocation (5,6). In a study of the gibberellin-enhanced phytotoxicity of bentazon (3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide) and glyphosate (N-(phosphonomethyl) glycine), Sterrett and Hodgson (19) reported that the enhanced phytotoxicity to Canada thistle (Cirsium arvense (L.) Scop.) may be due to changes in acropetal translocation and accumulation in leaf sinks.

Fluazifop-butyl, a recently developed herbicide, is principally used for post-emergence control of annual and perennial grasses in broadleaf crops (1). When applied post-emergence, it moves throughout the plant in both the xylem and phloem but accumulates primarily in meristematic regions where it appears to interfere with ATP production (15,16).

It has been reported that the addition of gibberellic acid to the spray mixture alters the phytotoxicity of johnsongrass to fluazifop-butyl (12). It was the objective of this study to determine what the influence of gibberellic acid might be with regards to absorption and translocation of fluazifop-butyl in johnsongrass. It was found that the addition of gibberellic acid to the treatment resulted in a greater acropetal translocation of the radionuclide, as has been reported with other herbicides.

Materials and Methods

Plant Culture. Three-node johnsongrass rhizome pieces were planted in 2 L pots filled with a mixture of soil:sand:peat moss (4:1:1 v/v/v),
in a controlled environment chamber set for 30°C days and 24°C nights on a 16 h photoperiod. Lighting was supplied by fluorescent and incandescent lamps at an intensity of 30 klx. The relative humidity fluctuated between 60 to 70%. Chemical treatments were applied with a micropipet to a predetermined area on the upper surface of the median region of the youngest fully expanded leaf of johnsongrass plants in the six-to-seven leaf stage.

Chemical Treatment. Technical 1-14C-fluazifop-butyl and 3H-gibberellic acid (GA3) were obtained with specific activities of 21.5 mCi/mmol and 20 Ci/mmol, respectively. Stock solutions of 14C-fluazifop-butyl, dissolved in 50% methanol, and 3H-gibberellic acid dissolved in 100% methanol, containing 1.49 X 10^-2 uCi/ul and 3.85 X 10^-2 uCi/ul, respectively, were prepared. Treatments were applied to a 1.45 cm^2 area outlined with lanolin at an application rate equivalent to 0.2 kg/ha of fluazifop-butyl. This was determined by considering the uCi being applied to the area, specific activity, and molecular weight of the radionuclide being used and doing the necessary calculations to arrive at the 1.45 cm^2 area to be treated which would give the 0.2 kg/ha fluazifop-butyl rate. Treatment solutions were prepared with one vial containing 14C-fluazifop-butyl and water and another vial contained 14C-fluazifop-butyl, 3H-gibberellic acid, and non-labelled gibberellic acid. The non-labelled gibberellic acid was required to achieve the desired application rate equivalent to 7.0 g/ha. A crop oil concentrate was added to the solution at a rate of 1%(v/v). A microliter pipet was used to apply a total volume of 25.9 ul which contained 0.16 uCi of 14C-fluazifop-butyl with or without 0.5 uCi
of $^3$H-gibberellic acid.

Assay Procedures. Plants were assayed 24, 48, and 96 hours after treatment. They were separated into treated area, treated leaf, treated leaf meristem, apical parts (those plant parts above the treated leaf meristem), basal parts, and root fractions. Prior to plant fractionation the treated area was dipped in 10 ml of 100% methanol for 10 seconds to remove any $^{14}$C or $^3$H present on the surface of the treated area. Plant fractions were then frozen in a dry ice-methanol bath, lyophilized for 48 h, and combusted. Liquid scintillation spectrometry was utilized for $^{14}$C and $^3$H quantification of the plant fractions. Counts per minute were corrected for dilution, background, and oxidizing and counting efficiencies then converted to disintegrations per minute. These values were converted to percentages of the total recovered then subjected to analysis of variance, with the mean separation being done according to Fisher's LSD procedure. There were three plants per treatment and the experiment was duplicated.

Results and Discussion

Absorption and Translocation of $^{14}$C-label. Partitioning of the $^{14}$C label from fluazifop-butyl among the various fractions of plants not treated with gibberellic acid indicated that over 90% of the $^{14}$C-label applied remained in the treated area (Table 1). The label recovered in this fraction plus that recovered as treated area wash accounted for 95.2% of the total recovered $^{14}$C-label after 34 h (92.9 and 2.3, respectively) and can be considered non-translocated. Distribution among the other plant fractions after 24 h of incubation indicated that most of the remaining 4.8% of recovered $^{14}$C-label, most
Table 4. Percent of the applied 14-C fluazifop-butyl label recovered, inspecified plant fractions, after 24, 48, and 96 hours without gibberellic acid application.

<table>
<thead>
<tr>
<th>Location of 14-C Label</th>
<th>Incubation Period (%)</th>
<th>LSD0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hrs.</td>
<td>48 hrs.</td>
</tr>
<tr>
<td>Non-translocated 14-C label:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated Area Wash</td>
<td>2.3</td>
<td>1.7</td>
</tr>
<tr>
<td>Treated Area</td>
<td>92.9</td>
<td>92.6</td>
</tr>
<tr>
<td>Translocated 14-C label:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated Leaf</td>
<td>3.1</td>
<td>3.0</td>
</tr>
<tr>
<td>Treated Leaf Meristem</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Apical Portion</td>
<td>0.8</td>
<td>2.0</td>
</tr>
<tr>
<td>Basal Portion</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Roots</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>LSD0.05</strong></td>
<td>0.33</td>
<td>0.72</td>
</tr>
</tbody>
</table>
of it remained in the treated leaf. The next greatest concentration was in the apical portions above the treated leaf. The remaining label was partitioned somewhat equally in the lower fractions of the johnson-grass plants.

Very little difference was seen, with respect to non-translocated and translocated $^{14}C$-label partitioning between values at $24$ h and $48$ or $96$ h incubation. More that $94\%$ of the recovered label was found in the non-translocated plant fractions after $48$ h of incubation. The treated leaf was still the site of greatest percent translocated label recovered, followed by the apical portions of the plant. The remaining label was again equally distributed in the other three plant fractions. Plants analyzed $96$ h following treatment resulted in $91.8\%$ of the recovered label in the non-translocated state. Of the remaining $8.1\%$ identified as translocated $^{14}C$-label, the amount recovered in the apical plant fraction was equal to the amount found in the treated leaf and not less as had been seed following $24$ and $48$ h of incubation.

In comparing the plant fractions over time periods of incubation it was found that there were no differences between $24$ and $48$ h in the amount located in the treated area and treated area wash. However, following $96$ h there was a significant decrease in these areas. A similar response was found in percent recovery in the treated leaf, except that there was an increase in the label rather than a decrease. No changes were seen in movement into the treated leaf meristem over time. In the apical portion there was an increase in the $^{14}C$-label over time at all three incubation periods. In basal and root fractions there was a decrease at $48$ h but after $96$ h it had increased back to
the level obtained after 24h.

Distribution of the $^{14}$C-label from fluazifop-butyl with the addition of gibberellic acid (Table 2) were similar to those obtained without gibberellic acid. An analysis of the plant following 24 h incubation showed 95.1% of the $^{14}$C-label remaining in the non-translocated fractions (1.9 plus 93.2% for treated area wash and treated area, respectively), which left 4.9% of the label classified as translocated label. This translocated portion was partitioned with the treated leaf receiving three times as much label as the apical portion, which had received greater than three times the amount recovered in the remaining plant fractions.

At 48 h after treatment 93.5% of the recovered label was still non-translocated. Of the remaining 6.5% which was translocated, equal amounts were recovered in the treated leaf and plant parts apical to the treated leaf meristem. The remaining label was equally distributed in the lower fractions. When johnsongrass was analyzed 96 h after treatment, with the addition of gibberellic acid, 93.2% of the $^{14}$C-label did not leave the treated area. Translocation from the site of application was 6.8% and distributed itself similar to the pattern found for the 48 h analysis.

Different plant fractions demonstrated different responses over time. With the treated area wash there was a decrease in the amount recovered after 96 h and this was different from what was found with the treated area and root portions, here there was a decrease after 48 h but no further decrease was observed after 96 h. The same trend was observed for the apical portion except it involved increases rather
Table 5. Percent of the applied 14-C fluazifop-butyl label recovered, in specified plant fractions, after 24, 48, and 96 hours with an exogenous gibberellic acid application.

<table>
<thead>
<tr>
<th>Incubation Period</th>
<th>Location of 14-C Label</th>
<th>24 hrs.</th>
<th>48 hrs.</th>
<th>96 hrs.</th>
<th>LSD₀.₀₅</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td></td>
</tr>
<tr>
<td>Non-translocated 14-C label:</td>
<td>Treated Area Wash</td>
<td>1.9</td>
<td>1.4</td>
<td>0.8</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>Treated Area</td>
<td>93.2</td>
<td>92.1</td>
<td>92.4</td>
<td>0.59</td>
</tr>
<tr>
<td>Translocated 14-C label:</td>
<td>Treated Leaf</td>
<td>3.3</td>
<td>2.9</td>
<td>3.2</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>Treated Leaf Meristem</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Apical Portion</td>
<td>1.0</td>
<td>2.9</td>
<td>3.1</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>Basal Portion</td>
<td>0.2</td>
<td>0.3</td>
<td>0.2</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Roots</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>LSD₀.₀₅</td>
<td>0.58</td>
<td>0.65</td>
<td>0.23</td>
<td></td>
</tr>
</tbody>
</table>
than decreases after 48 h. No differences were found in the treated leaf fraction over time. It was seen, with the treated leaf meristem and basal portion, that an increase in recovery occurred after 48 h but that the 96 h reading decreased to the same level as was found after 24 h incubation.

Partitioning of the $^{14}$C-label in various plant fractions with and without gibberellic was compared at the three incubation periods (Table 3) and indicated that after 24 h incubation no response was found when gibberellic acid was added. Comparisons of the plant fractions after 48 h of incubation showed more $^{14}$C-label was moved from the leaf surface yet this decrease in percent recovery from the treated area wash did not increase the amount of label recovered in the treated area itself. The areas of accumulation of this label as influenced by the gibberellic acid application, were the treated leaf meristem and the apical portion of the plant. This is almost the direct opposite of what was found after 96 h. Here those plant fractions which demonstrated gibberellic acid effect were the treated leaf, basal portion, and roots, one which showed no response after 48 h, and here showed a decrease in the amount recovered as a result of the gibberellic acid application.

Absorption and Translocation of the $^{3}$H-gibberellic acid. The partitioning of the $^{3}$H-label from the gibberellic acid in various plant fractions in the presence of fluazifop-butyl indicated that, like the $^{14}$C-label from fluazifop-butyl, most of the $^{3}$H-label was located in the non-translocated fractions, treated area and treated area wash (Table 4). The non-translocated fractions accounted for
Table 6. Percent recovery of $^{14}$C fluazifop-butyl label after 24, 48, and 96 hours, in specified plant fractions, as influenced by an application of gibberellic acid.

<table>
<thead>
<tr>
<th>Location of $^{14}$C Label</th>
<th>Period of Incubation Following Application</th>
<th>24 hours</th>
<th>48 hours</th>
<th>96 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- GA + GA</td>
<td>LSD$_{0.05}$</td>
<td>- GA + GA</td>
<td>LSD$_{0.05}$</td>
</tr>
<tr>
<td>Non-translocated $^{14}$C label:</td>
<td></td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>Treated Area Wash</td>
<td>2.3</td>
<td>1.9</td>
<td>1.21</td>
<td>1.7</td>
</tr>
<tr>
<td>Treated Area</td>
<td>92.9</td>
<td>93.2</td>
<td>0.97</td>
<td>92.6</td>
</tr>
<tr>
<td>Translocated $^{14}$C label:</td>
<td></td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>Treated Leaf</td>
<td>3.1</td>
<td>3.3</td>
<td>1.40</td>
<td>3.0</td>
</tr>
<tr>
<td>Treated Leaf Meristem</td>
<td>0.1</td>
<td>0.1</td>
<td>0.02</td>
<td>0.1</td>
</tr>
<tr>
<td>Apical Portion</td>
<td>0.8</td>
<td>1.0</td>
<td>0.43</td>
<td>2.0</td>
</tr>
<tr>
<td>Basal Portion</td>
<td>0.4</td>
<td>0.2</td>
<td>0.28</td>
<td>0.2</td>
</tr>
<tr>
<td>Roots</td>
<td>0.4</td>
<td>0.3</td>
<td>0.11</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Table 7. Percent of the applied 3-H gibberellic acid label recovered, in specified plant fractions, after 24, 48, and 96 hours.

<table>
<thead>
<tr>
<th>Incubation Period</th>
<th>Location of the 3-H Label</th>
<th>24 hrs.</th>
<th>48 hrs.</th>
<th>96 hrs.</th>
<th>LSD$_{0.05}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-translocated 3-H label:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treated Area Wash</td>
<td>48.1</td>
<td>46.9</td>
<td>39.2</td>
<td>1.55</td>
</tr>
<tr>
<td></td>
<td>Treated Area</td>
<td>47.5</td>
<td>48.2</td>
<td>52.2</td>
<td>1.55</td>
</tr>
<tr>
<td></td>
<td>Translocated 3-H label:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treated Leaf</td>
<td>2.5</td>
<td>2.6</td>
<td>4.9</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>Treated Leaf Meristem</td>
<td>0.2</td>
<td>0.2</td>
<td>0.3</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Apical Portion</td>
<td>1.0</td>
<td>1.3</td>
<td>1.7</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Basal Portion</td>
<td>0.4</td>
<td>0.5</td>
<td>0.8</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Roots</td>
<td>0.3</td>
<td>0.3</td>
<td>0.9</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>LSD$_{0.05}$</td>
<td>0.92</td>
<td>0.76</td>
<td>0.75</td>
<td></td>
</tr>
</tbody>
</table>
greater than 90% of the total recovered tritium label at all incubation periods. With the 24 h incubation the separation of the two components showed almost a 50:50 distribution of the label in the two. With the 48 and 96h incubation greater amounts were observed in the treated area as opposed to the treated area wash.

The partitioning of the remaining label resulted in twice as much moving into the treated leaf as was found in the apical portion or the remaining three fractions combined, regardless of incubation period. The change occurred in the amount of 3H-label leaving the non-translocated fractions and entering the translocation stream. The amount recovered in the translocated related process was essentially the same for the 24 and 48 h incubation and then doubled by the 96 h incubation.

The distribution over time, for all fractions indicated a shift in percent recovery, either decreasing as was the case with the treated area wash, or increasing as with the other fractions, when assayed after 96 h, compared to 24 or 48 h, yet there was no difference when comparing the 24 h with those values obtained after 48 h.

The distribution patterns of the 14C-labelled fluazifop-butyl indicated that following 24, 48, and 96 hours after application there was a limited amount of the label entering the translocation stream. That which moved out of the treated area moved throughout the plant. Rosser, et al. (16) reported that translocation of fluazifop-butyl occurred throughout the plant with areas of accumulation being young johnsongrass tillers, meristematic areas above the treated leaf, rhizomes and secondary shoots from rhizome connected to the treated
plant. The results of this study are in agreement with this and showed that, over time, there was a movement of the $^{14}C$-label into the apical portion of the plant. It was of interest to note that the reduction in percent recovery of label in the treated area wash was not reflected in increases in percent recovery in the treated area, but could have been the reason more label entered into the translocation process.

Little differences in the overall partitioning of the $^{14}C$-label were found when gibberellic acid was applied with fluazifop-butyl. There was still a greater percentage of the label in the non-translocated fractions and that which was translocated was moving out into the treated leaf, and primarily into the apical portions of the plant, while the rest of the label was distributed throughout the remaining fractions.

The influence of gibberellic acid on partitioning of $^{14}C$-labelled fluazifop-butyl was not observed until 48 h following application. It was found that more $^{14}C$-label was recovered in the apical portion and treated leaf meristem of those plants treated with fluazifop-butyl and gibberellic acid than with fluazifop-butyl alone. There was an increase in the amount leaving the surface of the leaf, suggesting enhanced absorption, though the mechanism whereby this is done is not known. The greater accumulation in the treated leaf meristem and apical portion of the plant could be explained if gibberellic acid acts by attracting photosynthates to an activated sink, as has been reported (20). This would result in the herbicide being brought along with
the photosynthates to the activated sink.

The enhanced acropetal translocation was not maintained for 96 hours. It is apparent that if gibberellic acid is enhancing acropetal translocation by stimulation to activated sinks, it is not doing so after 96 hours. There is, however, a decrease in the amount recovered in the treated leaf and basal and root portion. It is possible that this is the result of decreased entry from the treated area itself.

The distribution patterns of the $^3$H-labelled gibberellic acid, in the presence of fluazifop-butyl, showed very much the same pattern of partitioning as the $^{14}$C-labelled fluazifop-butyl except for the greater percent recovery in the treated area wash. Distribution over time would indicate that more gibberellic acid was available within the plant after 96 hours than at the 48 hour assay. If gibberellic acid was enhancing acropetal translocation of fluazifop-butyl by stimulating its movement to active sinks, the greater levels of gibberellic acid over time should have resulted in increased enhancement unless something was altering the physiological response of the applied gibberellic acid.

Murakami (14) demonstrated the general capacity of plant tissue to conjugate gibberellic acid. Barendse (3,4) observed the $^{14}$C GA$_3$ was metabolized in Pharbitis nil to a single product that had chromatographic and biological properties of $^{14}$C GA$_3$ glucosyl ether, with 83% being conjugated after 8 hours in light-grown seedlings. Schneider (17) recently reviewed the subject of gibberellin conjugation and indicated that gibberellic acid glucosyl conjugates represented the main group, that they were readily transported, and that the
glucosides, in general, show lower relative activities than glucosyl esters and they are, in fact, frequently inactive. It is possible that what is being seen with the decrease in acropetal translocation enhancement of the fluazifop-butyl is because the gibberellic acid is being conjugated and thus rendered inactive. This would also explain the greater distribution within the plant after 96 hours since conjugated gibberellins are more easily translocated compared to the free acid.


d-425.


CONCLUSION

The findings of the field screening experiment demonstrated that the use of gibberellic acid enhanced only one of the graminicides evaluated, fluazifop-butyl. The effects of the application of gibberellic acid with fluazifop-butyl as seen in the field, could be the result of increased translocation. Ashton (6) stated that gibberellic acid does not appear to affect the absorption of 2,4-D and that the increased effect is related to increased translocation which becomes evident after 3 to 5 days. The effects of gibberellic acid on the partitioning of photosynthates indicated that photosynthetic partitioning was altered with an increased movement to the acropetal parts of the plant (102, 202). This response is similar to what has been reported for gibberellic acid's influence on selected herbicides (14, 15, 209).

The visual response observed in the field would suggest that the results of adding gibberellic acid to the spray solution is through the greater accumulation of fluazifop-butyl in the apical growing points of johnsongrass. This would result in a greater visual phytotoxic response, as was found.

Few significant differences in the overall partitioning of the 14C-label were found when gibberellic acid was applied with fluazifop-butyl and was not observed until 48 hours following application. More radioactive label was recovered in the apical portions and treated leaf
meristem of those plants treated with fluazifop-butyl and gibberelic acid. Accumulation in those fractions was as expected if gibberelic acid were acting through stimulation of active sinks (220). This would result in greater accumulation of the herbicide, which is in the photosynthate stream, in the more active growing points of the plant.

The enhanced acropetal translocation was not maintained which indicates that if gibberelic acid were enhancing acropetal translocation by stimulation to activated sinks it was not doing so at 96 h. It would appear that something had happened which inactivated the physiological response. This may be what happened in the field when an initial increase in phytotoxicity was followed by a leveling off and stabilization of the effect.

One process which occur with gibberellins which result in an inactivation is conjugation (201,211). What is observed, possibly, is the initial gibberelic acid response followed by an inactivation of that initial gibberellin application by conjugation. This would help explain why even though there was greater amounts of gibberelic acid in the plant 96 h after application, the enhanced acropetal translocation was not maintained pass the 48 h incubation.

The next step in this area should be the determination of the state of the gibberellin at the various plant fractions and at different times. The enhancement of herbicidal phytotoxicity expression is an area that is full of alot of unanswered questions in which the role of the classic growth regulators could play a major role.
LITERATURE CITED


