THE UNIVERSITY OF MANITOBA

EFFECT OF BENZOYLPROP ETHYL AND BARBAN APPLIED ALONE OR COMBINED ON THE GROWTH AND DEVELOPMENT OF *Avena* SPP.

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF PLANT SCIENCE

WINNIPEG, MANITOBA

May 1975

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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ACKNOWLEDGMENTS

Dr. Elmer Stobbe, thank you for your advice, help, and criticism in this research project.

I appreciate the assistance and time of Dr. Punter and Dr. LaCroix given during critical developments of the project.

I am grateful to the following people for a helping hand; Laurel Coutts, Henry Nelson and Brian Hutton, and to Maeva.

Financial assistance was given by Shell Canada and the National Research Council.

Herbicides were donated by Gulf Research and Development Company and by Shell Canada Limited.

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University of Manitoba, 1975

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ABSTRACT

Field experiments were conducted to investigate the synergistic action of benzoylprop ethyl [ethyl-N-benzoyl-N-(3,4 dichlorophenyl)-2 aminopropionate], in combination with barban (4-chloro-2-butynyl-*m*-chloro-carbonilate), on wild oats (*Avena fatua*).

The treatments consisted of; barban alone, benzoylprop ethyl alone and the combination of the two herbicides. The treatments were applied at three different leaf stages of wild oats, the one-two, three-four, and the five-six leaf stage. The treatments were applied according to a simple factorial design for all three leaf stages of wild oats.

Control of wild oats at the one-two leaf stage with the combination of benzoylprop ethyl plus barban was equal to or better than control of wild oats with benzoylprop ethyl or barban alone. Control of wild oats at the three-four leaf stage with the combination was consistently better than the control of wild oats with barban or benzoylprop ethyl alone. Control of wild oats at the five to six leaf stage with benzoylprop ethyl was not improved with the addition of barban.

The laboratory experiments were conducted to explain the synergistic activity that barban plus benzoylprop ethyl in combination showed

in the field experiments. Laboratory experiments were conducted to investigate the histological and cytological effects of barban, benzoylprop ethyl, and the combination of the two herbicides, on wild oat plants.

Wild oat plants were treated at the two leaf stage with benzoylprop ethyl and barban, alone or in combination. Two weeks after treatment, (after the development of the second internode of untreated wild oat stems), wild oat stems were; measured, sectioned, stained and photographed for comparisons of internode length and cell size.

Wild oat stems were reduced in length and width by all treatments. Cell length was reduced by all treatments, but benzoylprop ethyl treatments appeared to reduce cell length more effectively than either barban or the combination treatments. Cell width was reduced by all treatments, but the combination of barban plus benzoylprop ethyl appeared to decrease cell width more than other treatments.

Laboratory experiments were also conducted to study the effect of barban, benzoylprop ethyl and the combination of the two herbicides, on mitotic tissue of oats (*Avena sativa*). Tame oats were used in this experiment to avoid the dormancy behavior of wild oats. Oat seeds were exposed to the above treatments in an acetone solution, dried for 24 hours, and germinated in petri plates for 96 hours. Squashes were made of oat coleoptiles.

Cell division was suppressed by barban treatments, appeared to be stimulated by benzoylprop ethyl treatments, and suppressed by the combination of barban plus the low concentration of benzoylprop ethyl. The latter treatment was more effective than the barban treatment.

The apparent synergism that occurred with the combination of benzoylprop ethyl plus barban in the field experiments could not be attributed to either reduced cell length or cell division singly. The synergism may be due to reduction of both cell length and cell division.

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INTRODUCTION

Competition from wild oats will depress wheat yields. Fifty-four wild oat plants per square meter have been considered a moderate infestation, but as few as twelve wild oats per square meter can cause a significant loss in wheat yields (Bowden and Friesen, 1967). It has been estimated that 17.5 million hectares of grain crops in western Canada are infested with wild oats, and 75% of that has a moderate or heavy infestation (Alex, 1966).

Barban, a wild oat herbicide became commercially available in 1961. This herbicide was the only post emergent herbicide for wild oats in wheat until 1972, when benzoylprop ethyl was introduced. Barban gives effective control at the one to two leaf stage of wild oats, whereas benzoylprop ethyl will control wild oats most efficiently after the four leaf stage.

An ideal herbicide would provide control of wild oats over several growth stages, and be non-phytotoxic to many crops. At the present time, chemical control of wild oats at the three to four leaf stage is not adequate. A mixture of barban and benzoylprop ethyl may have interaction effective at this intermediate stage.

Field experiments were conducted to study the effect of the barban-benzoylprop ethyl mixture over several growth stages of wild oats. Experiments were also conducted to study the histological and cytological effects of barban, benzoylprop ethyl, and the mixture of the two herbicides to show why the mixture of barban and benzoylprop ethyl is effective.

LITERATURE REVIEW

Competition

Research has been carried out to determine the density of wild oat plants that will reduce crop yields. In all cases wheat yields declined drastically as wild oat densities increased (Friesen and Shebeski, 1960; Bowden and Friesen, 1967; Bell and Nalewaja, 1968).

Friesen and Shebeski (1960) found that 12-48 wild oat plants per square meter will reduce yields in a grain field. In the same study they found that at a level of 60 wild oat plants per square meter, yield depressions occurred in 89% of the fields tested.

Bowden and Friesen (1967) in their experiments found that wheat yields were reduced by as few as 12 wild oat plants per square meter under low fertility, but no reduction in wheat yields occurred with wild oat densities less than 70 wild oat plants per square meter, also under low fertility, a year later. They also found that wild oats influence wheat growth very early. Significant yield reductions had occurred in wheat before wild oats were removed, as early as the 2 leaf stage. They found that once wild oats reached a density of 120 wild oat plants per square meter, fertilized or non-fertilized, wheat yielded equally. The wild oat population seems to nullify the effects of fertilization.

Bell and Nalewaja (1968) found that at two different locations in North Dakota yield reductions varied under the same wild oat densities. They found that yields of wheat under fertile conditions were more reduced than under non-fertile conditions, and concluded that wild oats prevented the crop from fully utilizing the fertilizer. They stated that the exact density of wild oats that cause yield reductions of economic importance, is dependent upon the environment.

Chemical Control

To reduce the effects of wild oats on wheat yields, chemicals can be applied which decrease the competitiveness of the wild oats. Two post emergent herbicides which reduce yield depressions caused by wild oats are barban and benzoylprop ethyl.

Barban was first field tested in Western Canada in 1958 (Hoffman *et al.*, 1960). Satisfactory control of wild oats is possible with 0.28 kg/ha of barban when applied at the 1-2 leaf stage of wild oats (Hoffman *et al.*, 1960; Friesen, H. A., 1959; Holms and Pfieffer, 1962). Friesen, H. A. (1961) indicated that the time of application of barban was important. The optimum stage of application, when wild oats are most sensitive, is at the 1 1/2 - 2 1/2 leaf stage. At the three leaf stage of wild oats, tillering and reduced growth of the wild oats occurs, but fewer plants are killed.

Friesen, G. (1967) found that the optimum time of barban application was closely correlated to days after emergence of wild oats. Four to fourteen days after emergence, wild oats seemed to be most physiologically susceptible — this time corresponds to the 1 1/2 - 2 1/2 leaf stage of wild oats.

After this susceptible stage, higher rates of barban (1.12 kg/ha) were required to achieve the same control. Friesen also found that this high rate causes injury to wheat and that this injury was pronounced 15 days after emergence. The rate of 0.28 kg/ha causes similar injury 15 days after emergence.

Friesen, H. A. (1961) similarly concluded that the high rate (1.12

kg/ha) of barban required to control wild oats at the two and one-half to three leaf stage, had damaged the wheat, resulting in no increase in grain yield.

Benzoylprop ethyl was first field tested in western Canada in 1969. Selective action on wild oats in wheat was demonstrated by Bowden (1969). Several researchers determined the optimum rate and stage of an application of benzoylprop ethyl (Bowden, 1969; Cushon, 1969; Bowden *et al.*, 1970; Holm, 1972). They concluded that the optimum stage of application was the five to seven leaf stage of wild oats.

Bowden (1969) used 5.6 kg/ha of benzoylprop ethyl at the one to two leaf stage of wild oats, which did not give adequate control; 3.36 kg/ha of benzoylprop ethyl at the three to four leaf stage gave some control, but 1.12 kg/ha of benzoylprop ethyl at the five - six leaf stage of wild oats gave excellent control.

Bowden *et al.* (1970) correlated the stage of wild oats at the optimum time of application of benzoylprop ethyl to the development of the crop. They found that 1.5 kg/ha of benzoylprop ethyl applied at the commencement of crop tillering, and the appearance of the nodes gave one hundred per cent wild oat control.

Holm (1972) found that the rate of application was inversely proportional to the stage of application. He found that 1.68 kg/ha of benzoylprop ethyl applied at the four leaf stage of wild oats, gave equal control to 0.84 kg/ha applied at the 5 - 6 leaf stage. Over several leaf stages of wild oats, he found that control improved with later applications of benzoylprop ethyl.

Elimination of wild oat competition is reflected in greater yields of the crop when compared to a weedy check. To control wild oats, the optimum time for application of benzoylprop ethyl is at the 5 - 6 leaf stage; while the best yield response was obtained with earlier applications (Bowden, 1969; Cushon, 1969; Holm, 1972).

Holm (1972) found that the mean wheat yields from plots treated with benzoylprop ethyl at the 4 leaf stage of wild oats yielded higher than the plots treated at the 5 - 6 leaf stage. Holm concluded that benefits from increased wild oat control at the later stage, are offset by the longer duration of competition before treatment.

Bowden *et al.* (1970) found that yield increases of wheat from benzoylprop ethyl treatments were dependent on the initial density of the wild oat stand. The denser the wild oat stand, the greater the improvement of yield after application of benzoylprop ethyl.

Barban and Benzolyprop Ethyl Mixture

Several combinations of barban and benzoylprop ethyl were first tested in 1972 (Stobbe and Nelson, 1972). They indicated that wild oats could be controlled at the 3 - 4 leaf stage, using 0.14 kg/ha of barban, plus 0.56 kg/ha of benzoylprop ethyl, with no injury to wheat.

Mode of Action of Barban

Barban is a carbamate, and acts similar to other carbamate herbicides (Freed, 1966). Carbamates act as mitotic poisons (Ennis, 1947; Canvin and Friesen, 1959; Dubrovin, 1959; Morrison, 1962; Banting, 1970).

Dubrovin (1959) studied wild oat root tips after treatment with barban. He noticed groups of chromosomes without nuclear membranes or nucleoli.

Morrison (1962) found abnormal cells in wheat and barley seedlings that had germinated in a solution of diallate.[1] These cells were swollen and irregular, with polyploidy, scattered nucleoli and irregular shaped nuclei occurring.

Banting (1970) studied mitotic divisions of wheat and wild oats after treatment with diallate and triallate.[2] He found that the number of cell divisions decreased with increased diallate and triallate concentration. Chromosomal and nuclear abnormalities also occurred, such as thickened chromosomes, chromosome clumps and chromosome bridges. Nuclei were dumb-bell shaped and many polyploid cells were present.

Wheat was not affected to the same extent as wild oats, fewer abnormalities were prevalent. Banting also found that in wild oats, the mitotic abnormalities were caused by higher concentrations of herbicide, than the concentration that affected shoot length, and concluded that there was an effect on cell elongation at these low concentrations.

Kobayashi and Ishizuka (1974) reported that growth reduction of oats correlated with the inhibition of protein and ribo-nucleic acid synthesis, when treated with barban. Wheat was also affected, but recovered after three days.

Mode of Action of Benzoylprop Ethyl

Benzoylprop ethyl inhibits cell expansion and cell elongation (Holm, 1972; Jeffcoat and Sampson, 1973; Jeffcoat and Harries, 1973).

Holm (1972) studied with oat plants treated with 1.12 kg/ha of

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[1] S -2,3 - dichloroallyl N, N-diisopropylthiolcarbamate.

[2] S 2,3,3-trichlorallyl N, N-diisopropylthiolcarbamate.

benzoylprop ethyl. He found that the cells of treated wild oats were shorter than the untreated plants, and concluded that the repression of stem length was due to the repression of cell elongation.

Jeffcoat and Sampson (1973) found that a foliar application of benzoylprop ethyl to cereals grown in culture, caused reduction of extension growth of oats, through an inhibition of cell expansion. There was little effect on wheat.

Jeffcoat and Harries (1973) found that wild oat plants treated with benzoylprop ethyl showed reduction in cell elongation in both stem and leaves. Again wheat cells failed to show any effect from treatments.

Holm (1972) also noticed that the stem diameter of treated wild oats was decreased, and that the stem wall was thinner. Vascular bundles of treated plants were more numerous, smaller and less organized than untreated plants.

SECTION I

THE CONTROL OF WILD OATS IN WHEAT WITH BENZOYLPROP ETHYL AND BARBAN ALONE AND IN COMBINATION

ABSTRACT

Field studies were conducted to investigate the synergistic action of benzoylprop ethyl [ethyl-N-benzoyl-N-(3,4 dichlorophenyl)-2 amino-propionate], in combination with barban (4-chloro-2-butynyl-*m*-chloro-carbonilate), on wild oats (*Avena fatua*).

The treatments consisted of; barban alone, benzoylprop ethyl alone, and the combinations of the two herbicides. The treatments were applied at three different leaf stages of wild oats, the one-two, three-four and the five-six leaf stage. The treatments were applied in a simple factorial design for all three leaf stages of wild oats.

Control of wild oats at the one-two leaf stage with the combination of benzoylprop ethyl plus barban was equal to or better than control of wild oats with benzoylprop ethyl or barban alone. Control of wild oats at the three to four leaf stage with the combination was consistently better than control of wild oats with barban or benzoylprop ethyl alone. Control of wild oats at the five to six leaf stage with benzoylprop ethyl was not improved with the addition of barban.

INTRODUCTION

Wheat yields decline with increased density of wild oats. Conversely, decreasing the density of wild oats will increase the yield of the infested wheat. Satisfactory control of wild oats is possible with 0.28 kg/ha of barban when applied at the 1-2 leaf stage of wild oats (Hoffman *et al.*, 1960; Friesen, H. A., 1959; Holmes and Pfeiffer, 1962). Four to fourteen days after emergence of the wild oats were found to be most physiologically susceptible to barban, this time period corresponds to the 1½ - 2½ leaf stage of wild oats. Fifteen days after emergence, barban at 0.28 kg/ha causes injury to wheat (Friesen, G., 1967).

Bowden (1969) found that 1.12 kg/ha of benzoylprop ethyl applied at the five-six leaf stage of wild oats gave excellent control. Holm (1972) found that the rate of application of benzoylprop ethyl was inversely proportional to the stage of application and that control improved with later applications of benzoylprop ethyl. To control wild oats, the optimum time for application of benzoylprop ethyl is at the 5-6 leaf stage; while the best yield response was obtained with earlier applications (Bowden, 1969; Cushon, 1969; Holm, 1972).

Stobbe and Nelson (1972) found that wild oats could be controlled at the 3-4 leaf stage, using 0.14 kg/ha barban, plus 0.56 kg/ha of benzoylprop ethyl, with no injury to wheat. Neither barban nor benzoylprop ethyl results in adequate wild oat control at this leaf stage.

METHODS AND MATERIALS

Field Experiments

Experiments were conducted at Carman, Manitoba on a field of wheat stubble. On May 4, 1973, the field was seeded with Neepawa wheat, with a press drill, and over seeded with wild oats, using a hand held cyclone broadcast spreader. The soil was Almasippi very fine sandy loam, which was maintained at the recommended fertility level of the soil testing laboratory, University of Manitoba.

The project was divided into 3 experiments. Each experiment consisted of twenty treatments, replicated four times in a simple factorial design. Each plot was 2.7 meters by 6.4 meters.

Spray treatments were applied with Tee Jet 650067 flat fan nozzles, giving 59.51 l/ha at 3.16 kg/cm^2 on a 2.14 meter boom bicycle sprayer. A 45^o nozzle angle was used, and the sprayer was operated at a speed of 94 m/min.

In Experiment I treatments were applied when the wild oats reached the 1-2 leaf stage on May 25, 1973. In Experiment II treatments were applied when the wild oats reached the 3-4 leaf stage on May 29, 1973. In Experiment III treatments were applied at the 5-6 leaf stage of wild oats, on June 12, 1973.

Assessments of weed control in the three experiments were made after heading of the crop. The wild oat density of each plot was estimated by counting the number of panicles of wild oats present in 2 quadrants of 0.5 by 0.5 meters. The effect of the treatments on seed set, was estimated by counting the wild oat seeds from plants which showed the effects of the spray treatments. Seeds were selected from 10 panicles in each plot, and averaged to give the number of seeds per panicle. Visual ratings of wild oat control, and crop tolerance from each plot, were compiled according to Table 1.

TABLE 1. EXPLANATION OF VISUAL WEED CONTROL AND CROP TOLERANCE RATINGS

Rating	Degree of Weed Control	Degree of Crop Tolerance
0	No control	No tolerance
1	Very poor control	Very severe stand reduction
2	Poor control	Severe stand reduction
3	Slight control	Moderate stand reduction
4	Moderate control	Slight stand reduction
5	Fair control	Plant height reduced
6	Nearly acceptable control	Slight deformity
7	Acceptable control	Acceptable tolerance
8	Very good control	High degree of tolerance
9	Complete control	Complete tolerance

Expected results for the combinations appear on Tables 2 through 16. These results were obtained by averaging the results from benzoylprop ethyl alone or barban alone at double the concentration of barban plus benzoylprop ethyl in combination. For example, the expected results for the combination of 0.56 kg/ha benzoylprop ethyl plus 0.14 kg/ha barban was determined by averaging the results of 1.12 kg/ha of benzoylprop ethyl and 0.28 kg/ha of barban.

The results from the panicle counts, and from seed counts per panicle were analyzed as square root transformed data ($\sqrt{\bar{x}}$).

Germination Experiment

The germination of wild oat seeds from plants affected by the herbicide treatments, in each experiment, was investigated. A sample of 20 seeds per plot was collected from the affected wild oat plants in each experiment. Due to the shattering characteristic of mature wild oat panicles, another sample of 20 seeds per plot was collected from the ground in each experiment. The plant seeds and the ground seeds were kept separate throughout the investigation. Each seed sample was weighed.

Surface sterilization of the ground seeds was carried out as follows:

- seeds were washed in a 2% Javex solution for 2 minutes.
- the Javex was decanted off, and the seeds were rinsed twice in sterile water.

Plant seeds were not surface sterilized.

The seeds were placed in plastic petri plates on 2 layers of #1 Whatman filter paper, in 5 ml of sterile H_2O. The plates were placed in a dark germination cabinet at a controlled temperature of $18 \pm 2^{o}C$ for 21 days. The plates were removed from the germination cabinet and placed in a cold cabinet at $2^{o}C$ for 21 days in order to break dormancy. The plates were removed from the cold cabinet, watered and placed in the germination cabinet for a final 21 day period.

Germinated seeds were recorded and discarded three times a week while germination took place.

RESULTS AND DISCUSSION

Due to uneven emergence, the wild oats showed considerable variation in growth and development. The first flush of wild oats emerged with the crop, but the majority of the wild oat stand emerged a week later. In Experiment I, the treatments were applied when the majority of the wild oats were in the two leaf stage. By this time many wild oats had passed the two leaf stage. Some wild oats emerged after the treatments were applied.

Experiment I. The effect of barban and benzoylprop ethyl alone, and in combination, on wheat and wild oat growth, 1-2 leaf stage of wild oats.

Wheat yields, from the treated plots, were not greater than the untreated plots unless 0.14 kg/ha of barban or 0.84 kg/ha of benzoylprop ethyl was applied (Table 2).

TABLE 2. WHEAT YIELD FROM PLOTS TREATED WITH BARBAN AND BENZOYLPROP ETHYL ALONE OR COMBINED - WILD OATS 1-2 LEAF STAGE

Benzoylprop ethyl kg/ha	Barban kg/ha			
	0.00	0.07	0.14	0.28
0.00	787	813	910	926
0.28	791	990 (845)*	929 (853)*	726
0.56	780	945 (932)*	964 (940)*	857
0.84	926	925	1046	835
1.12	954	1036	947	860

$LSD_{.05}$ = 116 *Expected yield.

When 0.28 kg/ha of barban was applied in combination with any rate of benzoylprop ethyl, the result was wheat yields not greater than the yields from the untreated plots (787 kg/ha). Plots treated with all other combinations of benzoylprop ethyl plus barban resulted in wheat yields which were greater than those from the untreated plots.

Barban applied at 0.28 kg/ha in combination with any rate of benzoylprop ethyl caused injury to wheat (Table 3), and subsequently reduced the potential yield of wheat (Table 2).

TABLE 3. THE EFFECT OF BARBAN AND BENZOYLPROP ETHYL, ALONE OR COMBINED, ON WILD OAT CONTROL AND CROP TOLERANCE - VISUAL ASSESSMENT, WILD OATS 1-2 LEAF STAGE.

Benzoylprop Ethyl kg/ha	Barban kg/ha							
	0.00		0.07		0.14		0.28	
	Wild oats	Wheat	Wild oats	Wheat	Wild oats	Wheat	Wild oats	Wheat
0.00	0	9	5	9	5	8	7	8
0.28	5	9	7 (4.5)*	9	6 (5.5)*	8	8	5
0.56	4	9	7 (6.0)*	8	8 (6.0)*	9	8	6
0.84	6	9	8	8	8	8	8	5
1.12	6	9	8	8	8	8	8	5

*Expected values.

The injury may have occurred because the majority of the wild oats emerged after the wheat, and application of the treatments was delayed until the wild oats reached the two-leaf stage. At the time of spraying, about fifteen days after emergence, the wheat had approached a critical time of susceptibility to barban (Friesen, G., 1967).

Satisfactory control of wild oats did not occur with any treatment of