8

EFFECT OF CARBOXIN ON LOOSE SMUT, *USTILAGO TRITICI*, OF WHEAT

by

ANWAR BAIG

B. Sc., University of Peshawar, Peshawar, Pakistan, 1965
M. Sc., University of Peshawar, Peshawar, Pakistan, 1967

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Plant Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1972

Approved by:

Earl D. Hansing
Major Professor

TABLE OF CONTENTS

INTRODUCTION

Loose smut of wheat is caused by Ustilago tritici (Pers.) Rostr. It infects the ovary during the flowering stage or shortly after fertilization. The mycelium develops in the growing embryo and then remains dormant in the scutellum of mature seed. The fungus does not usually cause visible symptoms in the wheat plant during the period of vegetative growth. Symptoms appear after emergence of black spikes.

Loose smut is an important and widely distributed disease all over the world and particularly where susceptible cultivars of wheat are grown. The annual loss in Kansas due to smut a decade ago was estimated at 7,000,000 bushels (10) although through control measures is currently less severe.

A systemic fungicide, carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) has been recently developed to control smut through chemical seed treatment. The chemical has a high degree of specificity for certain Basidiomycetes including U. tritici and U. nuda (Jens.) Rostr. (5, 33). Other workers (4) have demonstrated that 10 ppm carboxin prevents the germination of U. nuda teliospores.

The objectives of this study were to demonstrate the effect of carboxin on Ustilago tritici in vitro, and to demonstrate the effect of seed size and weight on the incidence of loose smut in wheat.

REVIEW OF LITERATURE

Fischer and Holton (8) reported that the life history of wheat loose smut was first investigated at the end of the last century by Maddox (1896). It was demonstrated that embryo infection took place only when teliospores were dusted on young ovaries during the pollination period.

Fischer and Holton (8) proposed that dormant mycelia in the embryos of wheat and barley seed were the source of loose smut in mature plants as proposed by Heck (1904). Histological studies revealed that the systemic fungus keeps pace with the growing point of infected cereal plants (9).

The presence of Ustilago tritici mycelia in the embryos of infected grain has been reported by many workers (35, 38, 41, 42). Klushnikova (22) studied the distribution of U. tritici mycelia in host tissue from the seedling stage to maturity, and the resulting anatomical changes in the host. She established the presence of mycelia in stem, leaf, and sheath tissue of diseased plants. She also found mycelia in roots, but only in the early stages of growth. These anatomical studies also revealed less fungal tissue in leaf than in stem sections. Hypertrophy existed in infected stem tissue, as intercellular spaces were enlarged within the vascular tissue. In addition, the superficial area of chlorophyll parenchyma was greater in infected plants. The number and size of stomata in leaf tissue also were increased.

Popp (34) found U. tritici mycelium in embryos, seedling meristems, and in spike primordia of mature plants. However, its presence in seedling meristems is the only positive means of forecasting losses in the mature plants.

The relation between embryo infection and losses (40) from loose smut encouraged the development of new and rapid methods to examine embryos for presence of mycelia (35, 41, 42). The methods, as simplified by Morton, are

now used as a routine test to determine the percentage of seeds with U. tritici infection (32).

The mycelium of the loose smut fungus systemically infects the embryo scutellum and therefore surface applications of nonsystemic fungicides cannot reach or control the fungus directly.

The new systemic fungicide, carboxin, has been highly effective as a seed treatment for the control of various seed and soil-borne pathogens. Von Schmelling and Kulka (44, 45) were the first to report that carboxin gives complete control of loose smut. Their results were soon confirmed by Edgington, Walton and Miller (5) and Hansing (11). Edgington (6) also indicated that carboxin is compatible with other organic fungicides. Many other workers have corroborated that carboxin seed treatments completely control loose smut of wheat and barley (1, 2, 12, 14, 15, 16, 19, 21, 24, 26, 30, 33).

Apparently no phytotoxicity results when seeds are treated with carboxin (13, 16, 29). Kiesling (19) reported a slight yield reduction when wheat or barley was treated with carboxin. However, Jones and Clement (16) did not find reduction of yield or phytotoxicity when plants were treated with carboxin. Edgington and Barron (4) reported selectivity of the carboxins for the Basidiomycetes.

Mathre (27) studied the uptake and binding of the oxathiin fungicide by resistant and sensitive fungi. He found that uptake of C-14 labeled carboxin by Rhizoctonia solani and Ustilago maydis was rapid and that maximum uptake occurred 30-90 min after initial exposure to the fungicide. In comparison Fusarium oxysporium f. sp. lycopersici and Saccharomyces cerevisiae absorbed very little carboxin after 5 hr. By exposing disrupted cells to carboxin, he found that 90-95% of the fungicide was associated with the ribosomal fraction. He concluded that specificity of the fungicide was related to the amount entering

the cell. Mathre also demonstrated the inhibition of aerobic respiration in sensitive fungi by treatment with carboxin (28).

Snel et al. (43) recently studied the fungitoxicity and structure-activity relationship of some oxathiin and thiazole derivatives. They stated that the oxathiins and other systemic fungicides act by altering the host's metabolism.

Fezer (7) indicated that the incidence of loose smut increased with decreasing seed size. McFadden et al. (25) also found that a higher per cent of small and medium-sized than large seeds were infected. In this study they used seeds from different regions, which were produced in different years and found the effect to be universal.

Krull and Robayo (23), working with 'Funza' barley, concluded that seed size was indirectly correlated with the per cent of smutted spikes. The effect of seed size and weight on yield has also been studied (3, 20, 46). Kiesselbach (20) has summarized much of the earlier work and stated that large seed generally produce greater yield than small seed. Studies by Kaufmann and McFadden (17) also indicated that large seed, on an average, produced more tillers than small seed. Demirlicakmak et al. (3) found that seed size had no effect upon emergence.

MATERIALS AND METHODS

Seed Source and Treatment

'Bison' wheat seed which had been artificially inoculated using Moore's method (31), was utilized throughout the course of this investigation. Approximately 66% of the seed inoculated in this manner was systemically infected with U. tritici as illustrated in Fig. 1 and 2.

The seed was divided into large and small classes by sieving through Tylar Standard screens. Seeds retained on the 8-mesh screen were considered large and those retained on the 10-mesh screen were considered small. The seeds in each class were then individually weighed and grouped into three weight classes.

Commercial fertilizer (20% nitrogen, 20% phosphoric acid and 20% potash) was added to plants grown in the greenhouse at the rate of 0.3 g per pot.

Carboxin was used as the seed treatment. The chemical was applied at the rate of 3 oz/100 lb of seed.

Effect of Carboxin on Germination and U. tritici Infection

Carboxin-treated and untreated small seeds were germinated in paper towels at 20 C in a moist chamber. Seventy-five seeds were planted between each set of paper towels, and moistened with 100 ml of water. Each experimental treatment was replicated 4 times. Five days after germination, the seeds were divided into 3 groups: those with coleoptiles 0.0-0.4 cm, those with coleoptiles 0.5-7.4 cm long, and those with coleoptiles 7.5-12.5 cm long. The percentage of infection in each group was determined by the method of Morton (32).

A greenhouse experiment was conducted to determine the effect of carboxin on seed germination and percentage of smutted spikes. The seeds were divided into 3 weight classes: 0.014-0.018, 0.019-0.023, and 0.024-0.028 g/seed. Four

THIS BOOK CONTAINS NUMEROUS PAGES THAT WERE BOUND WITHOUT PAGE NUMBERS.

THIS IS AS RECEIVED FROM CUSTOMER.

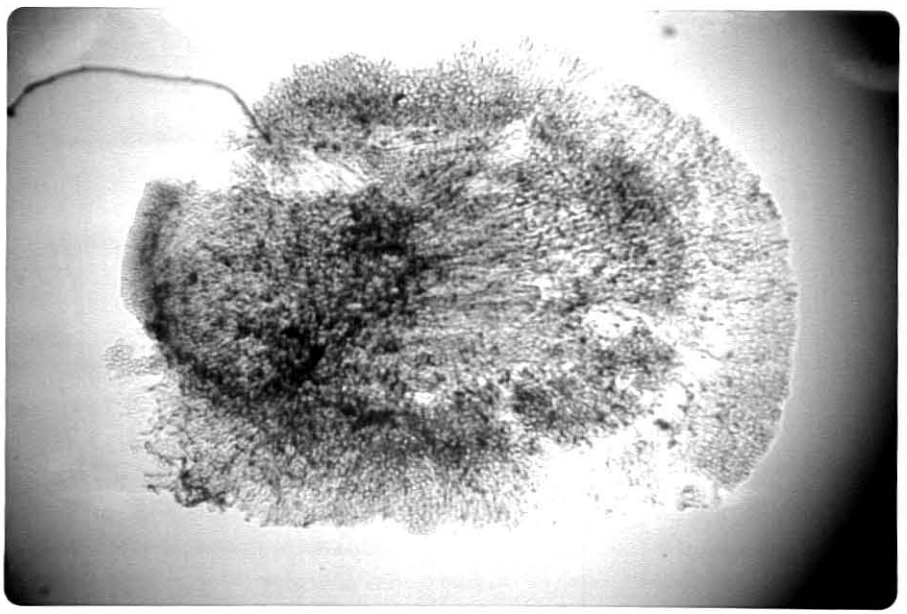
EXPLANATION OF FIG. 1

(i) Frontal view of infected embryo showing U. tritici mycelium, (x 50).

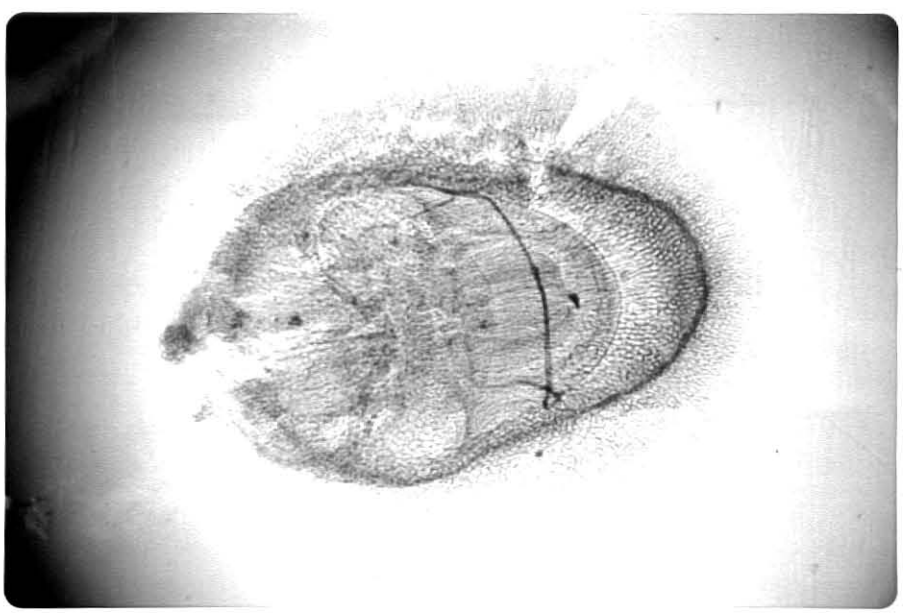
(ii) Frontal view of healthy embryo cleared in lactophonal, (x 50).

THIS BOOK CONTAINS NUMEROUS PICTURES THAT ARE ATTACHED TO DOCUMENTS CROOKED.

THIS IS AS RECEIVED FROM CUSTOMER.



(i)

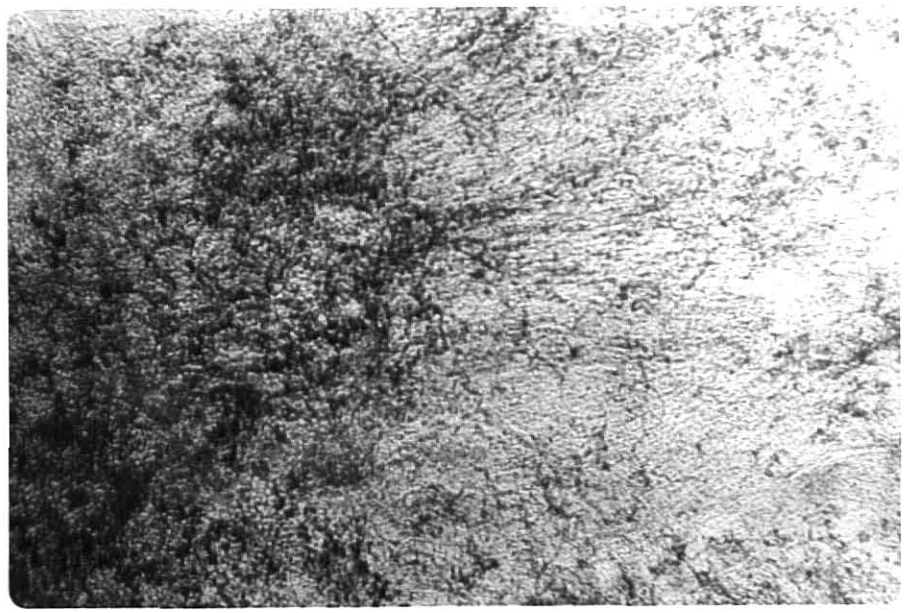


(ii)

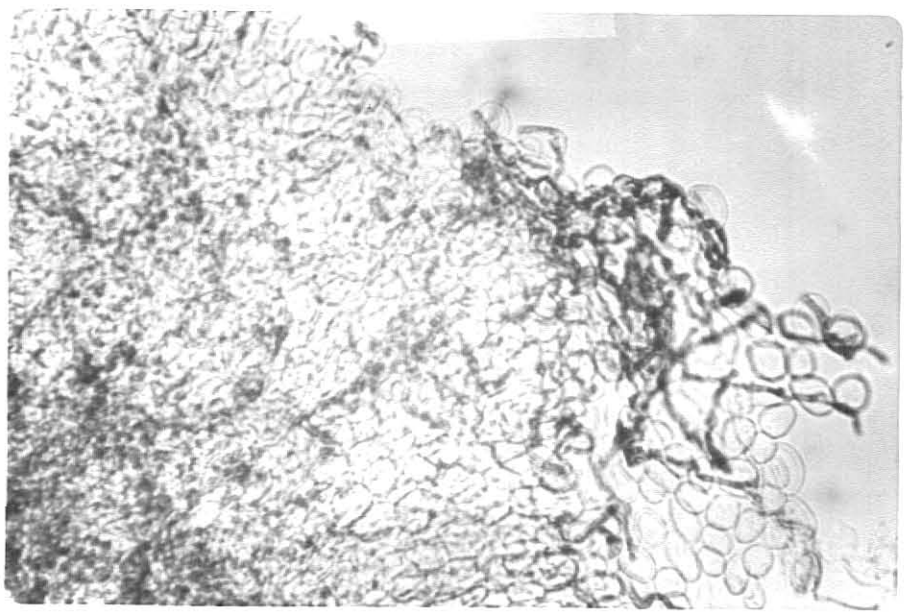
EXPLANATION OF FIG. 2

(i) Infected embryo tissue showing U. tritici mycelia, (x 100).

(ii) Infected embryo tissue showing U. tritici mycelia, (x 430).



(i)



(ii)

seeds of each weight class were then planted 2 cm deep in 15 cm plastic pots, and plantings of each weight class replicated 4 times. The pots were then kept at 4 C for 5 days to break dormancy and then randomized on greenhouse benches. Emergence counts were taken when the plants reached the two-leaf stage. Following this, plants were vernalized in a cold chamber for 40-50 days and then returned to the greenhouse for further growth. Smut percentages were determined at heading.

Effect of Seed Size and Weight on the Incidence of Loose Smut

Seeds were divided into large and small seed lots. The per cent of infection in each seed lot was determined by the embryo test, as illustrated in (Fig. 4, 5, 6) on 4 samples of 25 seeds each from each group.

Samples of 125 seeds from the large and small seed lots were randomly selected, the individual seeds weighed and the weights recorded. Four seeds of known weight, from large and small seed lots, were then planted at random in 15-cm clay pots. At maturity the total number of smutted and healthy spikes were recorded.

Effect of Carboxin on the Growth of U. tritici in Nutrient Solution

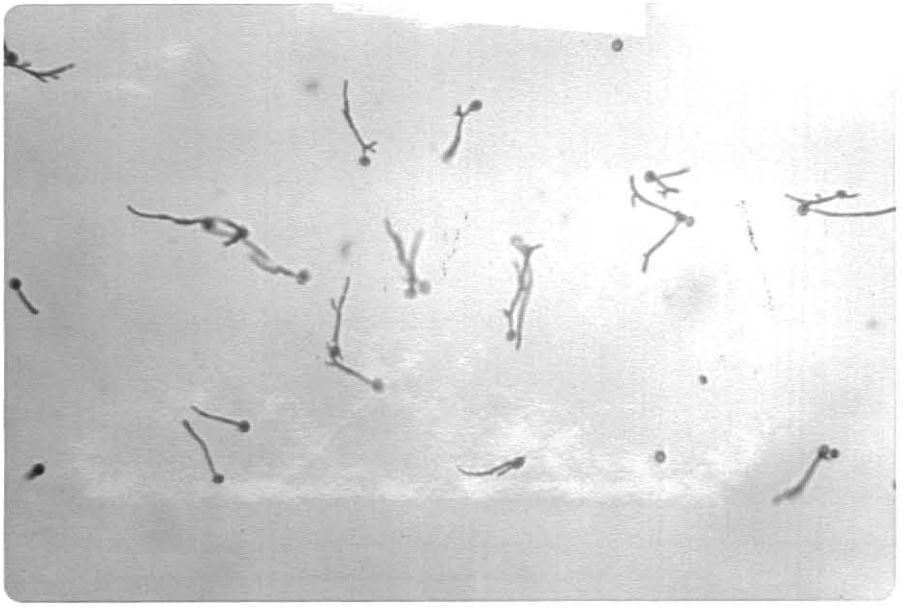
Experiments were conducted to see the effect of carboxin on the growth of U. tritici in culture. Teliospores of U. tritici were collected from smutted spikes and were washed for 10 to 15 minutes in 1% $CuSO_4$ solution containing one drop of Tween 20. The spores were then washed thoroughly with sterile distilled water and transferred to PDA (Fig. 3). After 4-5 days at room temp., the culture of U. tritici was transferred to 250 ml Erlenmeyer flasks containing the following medium:

Maltose 3 g, Malt extract 3 g, Potassium dihydrogen phosphate 1.25 g, Magnesium sulphate 0.3 g, Peptone 0.3 g, Distilled water 1 liter (39).

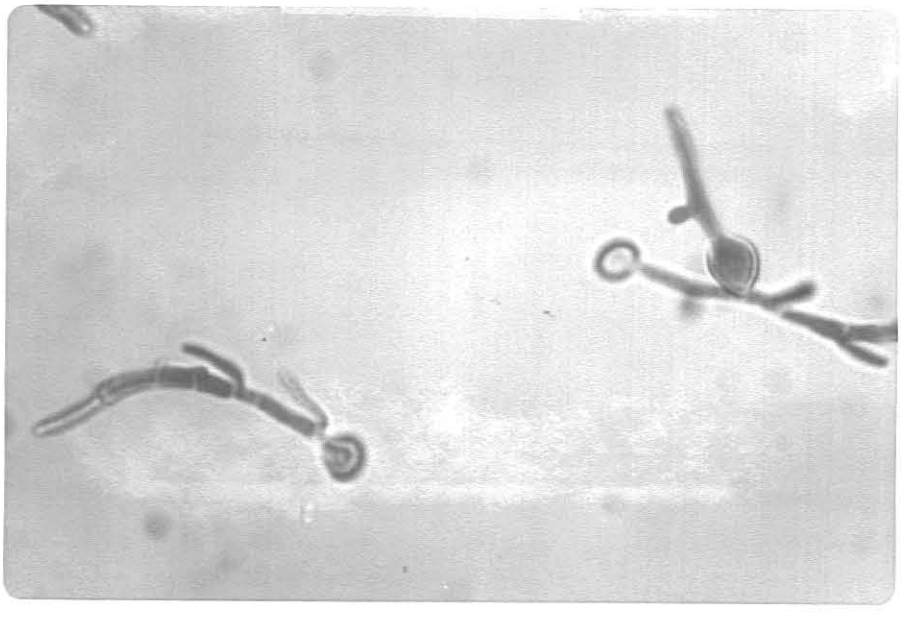
EXPLANATION OF FIG. 3

(i) Germinating teliospores of U. tritici, (10 x 10).

(ii) Germinating teliospores of U. tritici, (x 430).



(i)



(ii)

Flasks containing the fungus were then placed on a rotary shaker for one week at room temp. After this incubation period the mycelia-media mixtures were blended in a Waring Blendor. The inoculum necessary for further experimentation was then prepared by the addition of 30 ml of this blended material to 170 ml of sterile distilled water.

A stock solution containing 10,000 ppm carboxin was prepared by dissolving the fungicide in acetone. From this stock solution, petri plates each containing 20 ml of PDA were prepared so that the final carboxin concentrations were as follows: 100, 10, 8, 5, 2 and 0 ppm. Equal amounts of blended mycelium were then added to each plate and the plates incubated at 20 C for 5 days. After this period, the growth of each colony was measured.

In a second experiment, 2 ml of the blended mycelial suspension was added to 27.7 ml of nutrient solution in 250 ml flasks, and incubated for 10 days on a rotary shaker. Following this incubation, sufficient volumes of carboxin stock solution were added so that the final carboxin concentrations were as follows: 100, 10, 8, 5, 2 and 0 ppm. To check the effect of acetone used in preparing the stock solution upon U. tritici mycelium sterile deionized water (1% by volume) was added to several additional flasks as a control. After an additional 10 days of growth on the rotary shaker, the mycelia were removed, dried and weighed.

To check the fungistatic nature of carboxin, 2 ml of blended mycelium was added to 27.7 ml of nutrient solution in 250 ml flasks and incubated for 10 days. Carboxin stock solution was again added to make final concentrations as follows: 100, 10, 8, 5 and 0 ppm. The mycelia were removed after exposure periods of .5, 1, 2, 4, 8, 16 and 24 hr, and were either placed directly on PDA plates, or first washed for 10-15 min with sterilized distilled water and then placed on PDA. The plates were then incubated for 6 days at 20 C and the growth of the colonies was measured.

EXPERIMENTAL RESULTS

Effect of Carboxin on Germination and U. tritici Infection

When wheat seed was treated with carboxin, the number of germinated seeds and the per cent of infection was similar to that of untreated controls (Table 1). After germination, the seedlings were divided into 3 subgroups according to coleoptile lengths. An inverse relationship between coleoptile lengths and percentages of U. tritici infection occurred in plants from both treated and untreated seeds.

A greenhouse experiment was conducted to determine the effect of carboxin on seedling emergence and per cent smutted spikes. Carboxin treated and untreated seed were divided into 3 seed weight classes and 4 seeds from each weight class planted per pot. Carboxin did not affect the per cent germination, as both treated and untreated seeds germinated with the same frequency (Table 2). Likewise, seed size did not affect emergence, since small and large seeds from treated and untreated seed lots germinated equally.

Results of the embryo test indicated that 50% of the small seeds were infected with U. tritici. However, only 39% of the spikes grown from these seeds were smutted. At maturity the per cent of smutted embryos in large seeds was directly correlated with the number of smutted spikes produced.

Effect of Seed Size and Weight on the Incidence of Loose Smut

The incidence of smutted spikes, in plants grown from small seeds, was less than in plants grown from large seeds (Fig. 4, 5, 6). Regression analysis of the data showed no definite correlations between seed weights and smutted spikes (Fig. 7, 8).

Effect of Carboxin on the Growth of U. tritici in Nutrient Solution

Dry weights of U. tritici mycelia, which had been grown in nutrient

Table 1. Number of carboxin-treated and untreated seeds, with different coleoptile lengths after 6 days of germination in a 20 C moist chamber and per cent of seed infected in each coleoptile length group

Seed treatment	Coleoptile length (cm)	Number of seeds	Per cent of seeds infected
Carboxin	0.0 - 0.4	152	64
	0.5 - 7.4	91	62
	7.5 -12.5	57	40
None	0.0 - 0.4	138	69
	0.5 - 7.4	114	59
	7.5 -12.5	48	42

Table 2. Per cent smutted embryos, smutted spikes, and seedling emergences from carboxin-treated and untreated seed of 3 weight classes of Bison wheat

Seed-weight classes (g seed)	Per cent infected embryos	Per cent seedling emergence*		Per cent smutted spikes	
	Untreated	Carboxin	Untreated	Untreated	Treated
0.014-0.018	50	100	95	39	00
0.019-0.023	40	100	97	37	00
0.024-0.028	36	100	96	35	00

* Based on 96 seeds planted per treatment.

solutions containing various concentrations of carboxin, were all less than the untreated control (Table 3). As an additional control, dry weights were also obtained from U. tritici mycelia which had been grown in nutrient solution containing acetone as this solvent was used to make the carboxin soluble. Inhibition of mycelial growth was a factor of carboxin concentration only, and was independent of acetone concentration.

In a second experiment, the growth of U. tritici mycelium on PDA containing various concentrations of carboxin was observed. Only a trace of growth resulted on those plates amended with 2 ppm of carboxin and no growth occurred with higher concentrations (Table 3).

Uptake and Retention of Carboxin by U. tritici

The fungistatic nature of carboxin was demonstrated by the exposure of U. tritici mycelium to different carboxin concentrations for various lengths of time (Table 4). After treatment, the mycelium was either placed directly on PDA or first washed and then placed on PDA. After 6 days of incubation, diameters of the colonies were measured. Mycelial growth was observed to decrease with higher carboxin concentrations and longer exposures to time. Washed mycelium grew to a greater extent than unwashed mycelium regardless of initial carboxin concentrations. Only a trace of mycelial growth was observed after a 1/2 hr exposure to a concentration of 100 ppm carboxin. The growth of washed mycelium following the same carboxin treatment, was similar to that of untreated controls.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

THIS IS AS RECEIVED FROM CUSTOMER.

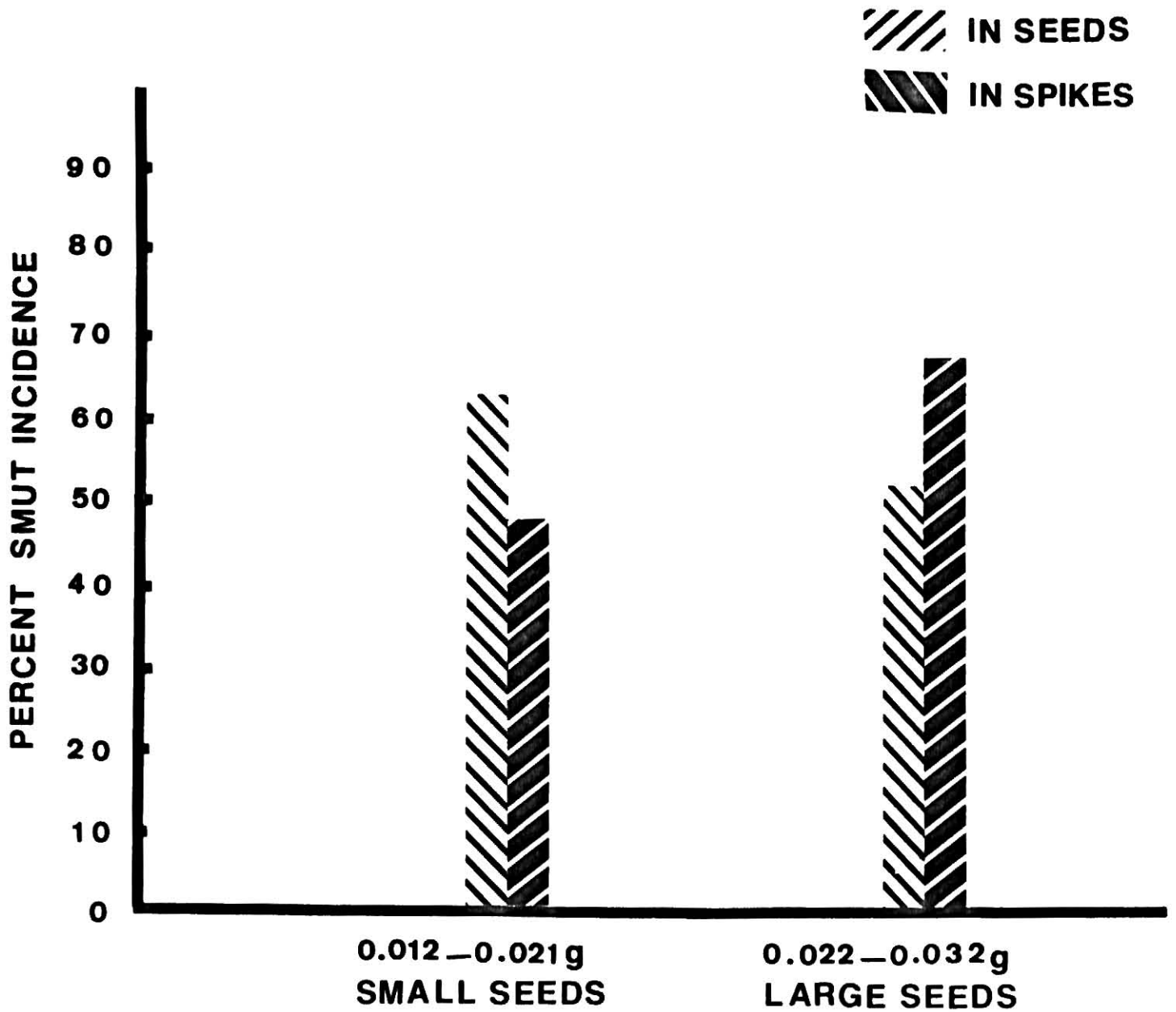


Fig. 4. Per cent smut incidence in seeds and spikes in small and large seeds.

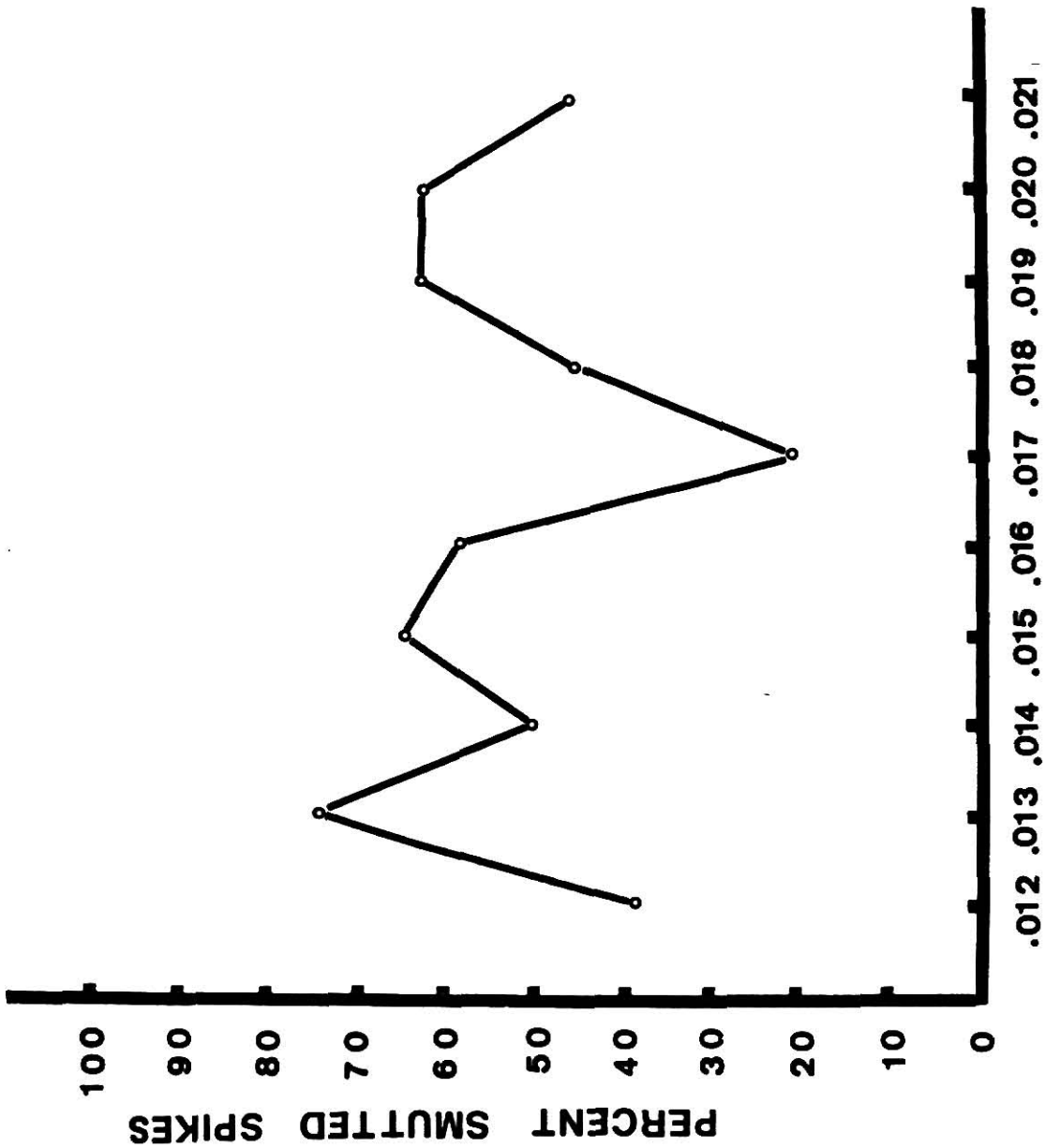


Fig. 5. Per cent smutted spikes from small seeds of different weights.

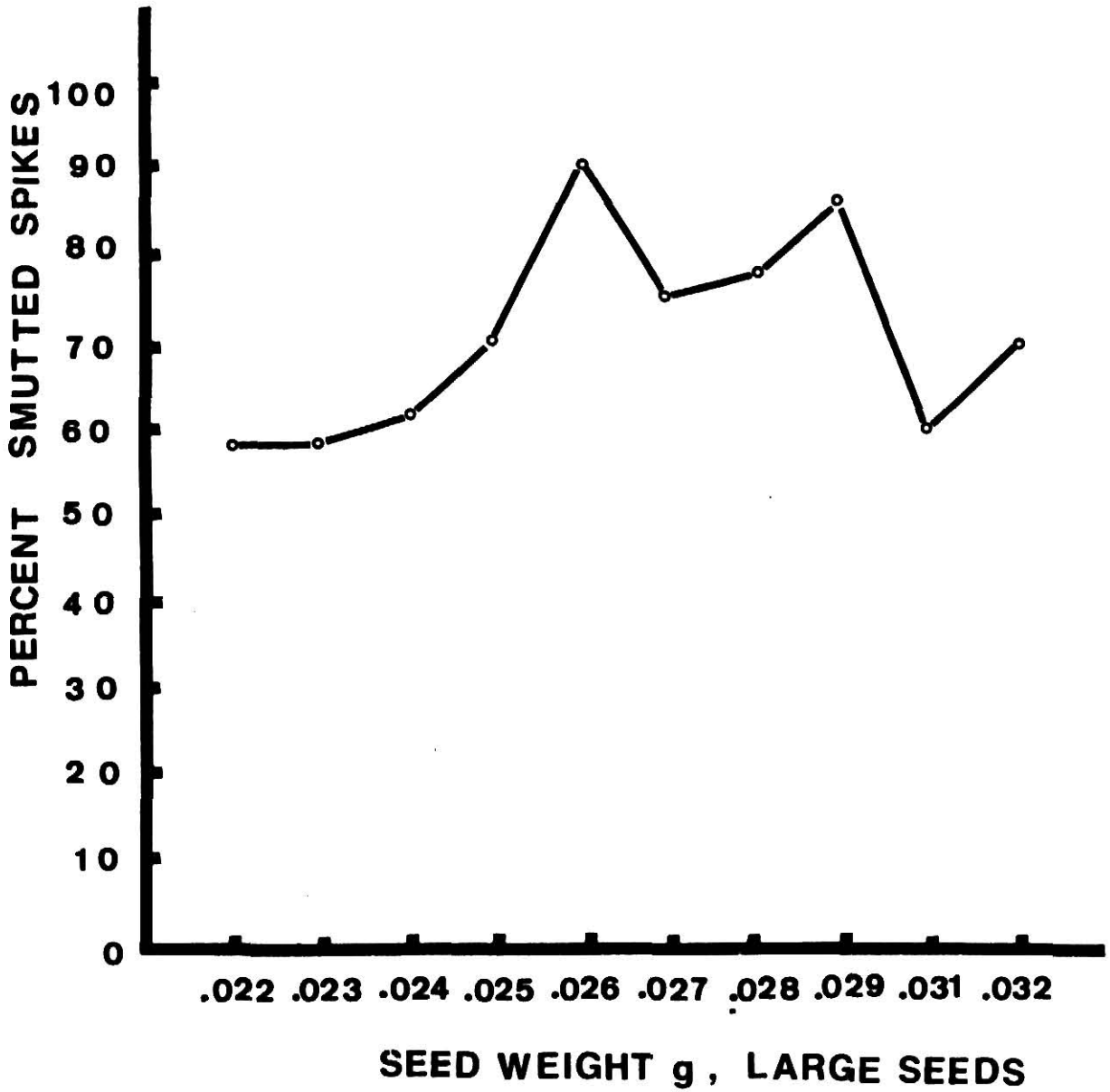


Fig. 6. Per cent smutted spikes from large seeds of different weights.

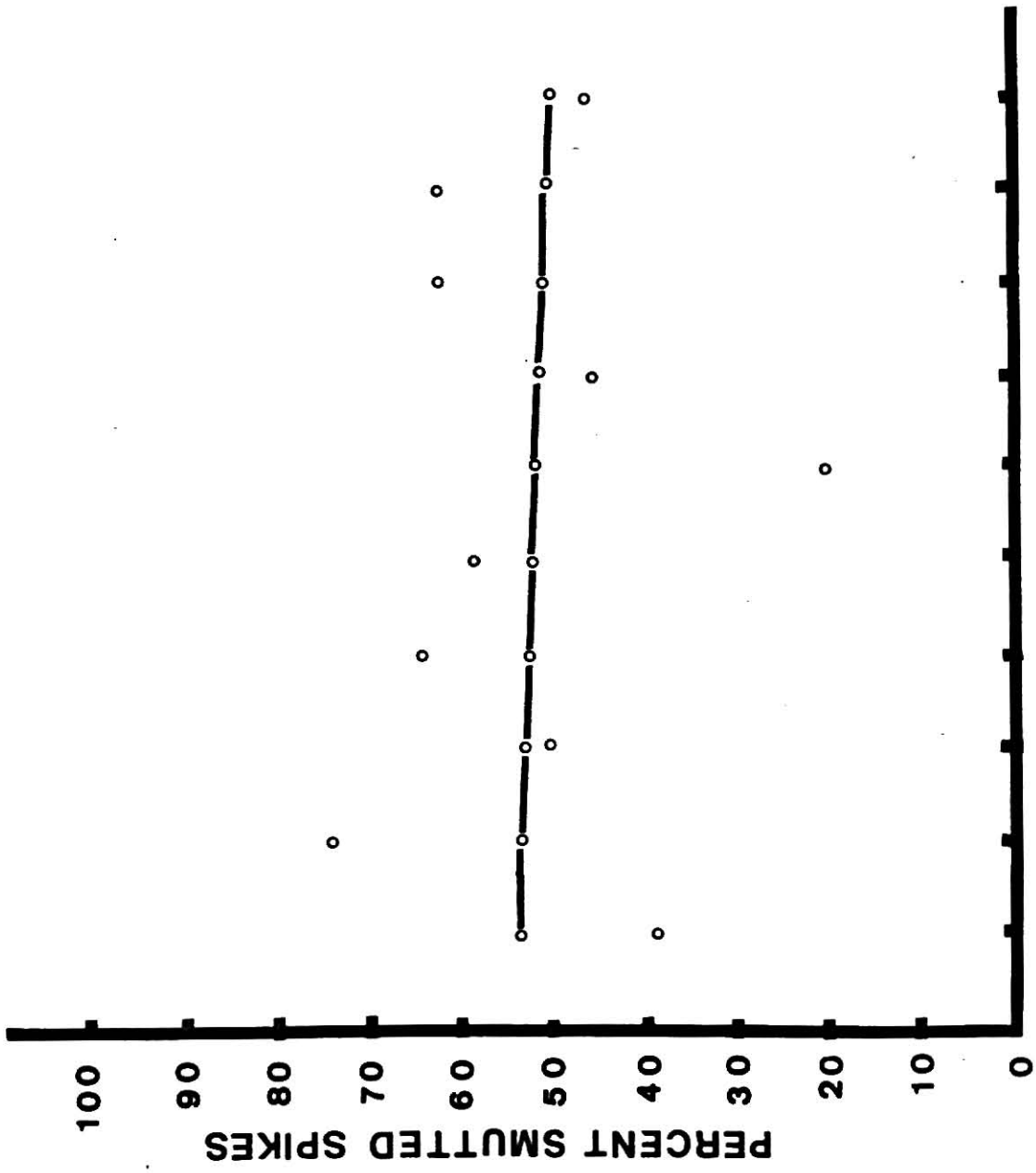


Fig. 7. Relation of seed weight to the incidence of smutted spikes.

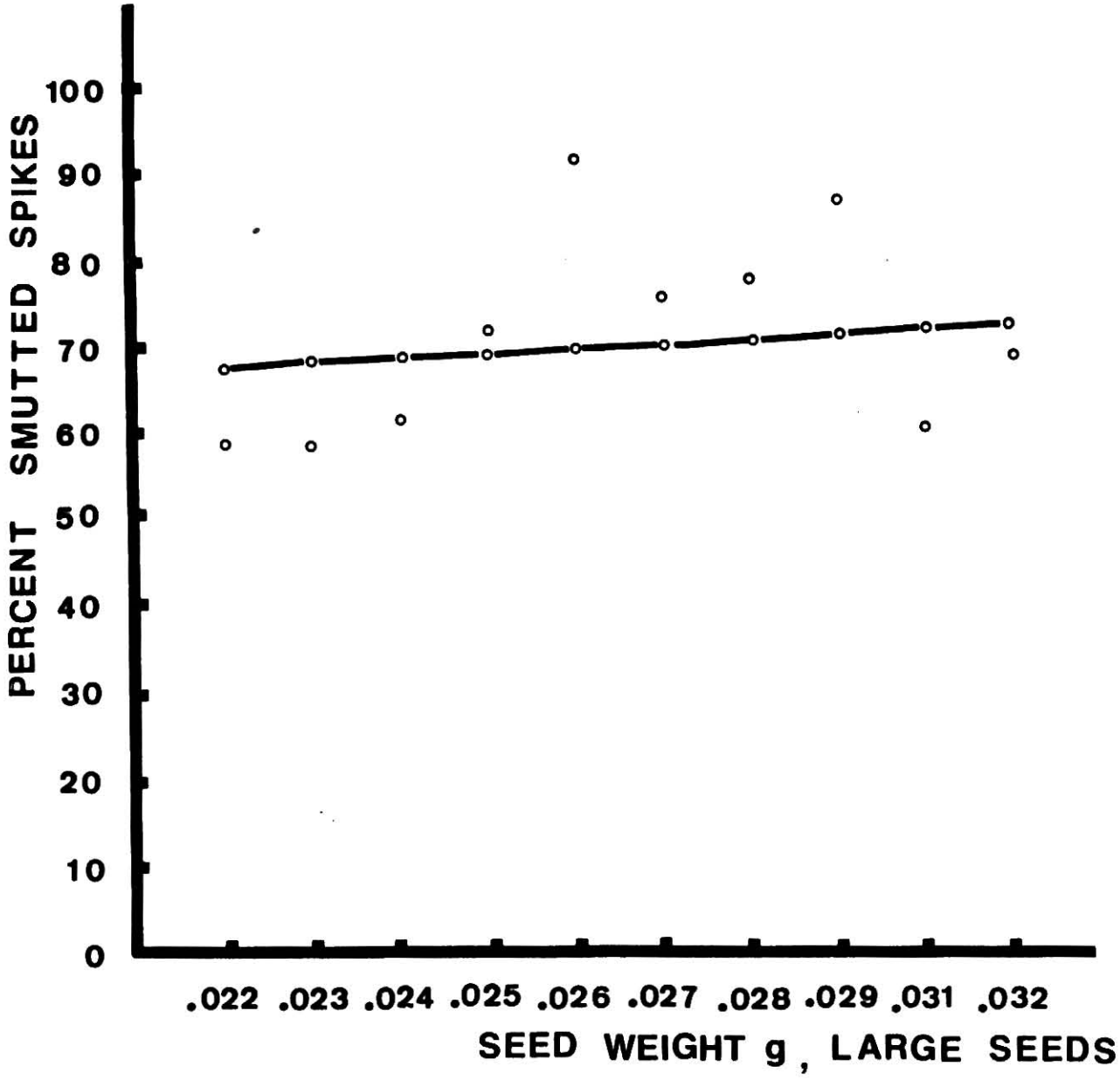


Fig. 8. Relation of seed weight to the incidence of smutted spikes.

Table 3. Growth of U. tritici mycelium after 6 days on carboxin amended PDA and dry weight of mycelium after 10 days in liquid shake cultures followed by 10 days growth in various concentrations of carboxin

Carboxin ppm	Colony diameter (mm) on PDA	Dry weight (g) in liquid nutrient media
100[1/]	0	0.006
10	0	0.006
8	0	0.008
5	0	0.012
2	Tr[2/]	0.018
0	4.0	0.030
0[3/]	4.0	0.028

1/ Concentrations of 100, 10, 8, 5, 2 and 0 ppm prepared by dissolving carboxin in acetone and adding to the media at the rate of 1% by volume.

2/ Trace of mycelial growth.

3/ One per cent by volume sterile distilled water added to nutrient media.

Table 4. Growth of U. tritici mycelium after 6 days on PDA following treatment of mycelium for various lengths of time in different concentrations of carboxin amended nutrient media

Carboxin ppm	Colony diameter (mm) Duration of carboxin treatment (hours) .5	1	2	4	8	12	24
			Mycelia washed following treatment				
100[1]	3.5	3.0	3.0	Tr[2]	1.0	0.0	0.0
10	3.0	3.0	3.0	2.5	2.0	Tr	0.5
8	3.0	2.5	2.5	2.0	2.0	1.5	0.5
5	3.5	3.0	3.0	3.0	2.5	2.0	1.5
0	4.0	4.0	3.5	4.0	4.5	3.5	3.5
0[3]	4.0	4.0	4.0	4.5	4.0	4.0	4.0
			Mycelia unwashed following treatment				
100[1]	Tr[2]	0.0	0.0	Tr	0.0	0.0	0.0
10	2.5	2.0	1.5	0.0	1.0	0.5	0.5
8	2.0	2.0	1.5	Tr	0.0	0.5	0.5
5	3.0	2.5	2.0	0.5	1.0	0.5	0.5
0	4.0	3.5	3.5	3.0	4.5	3.5	4.0
0[3]	4.0	3.5	4.5	4.0	4.0	5.0	4.5

1/ Concentrations of 100, 10, 8, 5 and 0 ppm prepared by dissolving carboxin in acetone and adding to the media at the rate of 1% by volume.

2/ Trace of mycelial growth.

3/ One per cent by volume sterile distilled water added to nutrient media.

DISCUSSION AND SUMMARY

Emphasis was directed in this study to determine the effect of carboxin on loose smut, U. tritici, of wheat. It would appear, from the data in Table 1, that infection was uniform within carboxin treated and untreated seeds. Apparently then, the fungicide becomes active only after the initiation of seedling growth. Possibly, with the advent of growth, carboxin is absorbed and then translocated to the area of fungal activity.

Greenhouse experiments indicated that small seeds were correlated with greater smut infection (Table 2). Russell (36) and Russell and Popp (37) found the percentage of smut in seed, as determined by the embryo test, to be highly correlated with the percentage of mature smutted plants grown from that seed. This study, however, indicated that this relationship was not necessarily true. When small seeds with 50% smut infection were planted, only 39% of the mature plants showed evidence of smut. When large seeds, containing 36% smutted embryos were planted, an equal percentage of smutted plants resulted (Table 2). The implication here is that a direct correlation exists only in the case of large seed.

Studies involving the germination of infected seed indicated that high seed infections resulted in lower germination rates (Table 1).

Several investigators (3, 17, 18, 20) have demonstrated greater wheat and barley yields with the planting of larger seed. Kaufmann and McFadden (17) found greater yield differences, from plants originating from large and small seed, when competition among plants was increased.

Seed weight as indicated in Fig. 7 and 8 had no significant effect on the incidence of loose smut in wheat at maturity. Although slight differences did exist, they were found to be insignificant upon a regression analysis of data.

The fungistatic nature of carboxin was also demonstrated in this study. When U. tritici mycelium was treated with 2 ppm carboxin, only a trace of growth was observed after 6 days.

In another experiment, when mycelium was washed and placed on carboxin free media, considerable growth resulted. Apparently, the washing was sufficient to reduce the carboxin concentration by dilution and therefore reverse the inhibition.

Edgington et al. (4) found that a concentration of 10 ppm carboxin was necessary to retard the germination of Ustilago nuda teliospores. In the present study, carboxin concentrations of only 2 ppm were sufficient to retard the growth of U. tritici mycelium.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to his major advisor, Professor Earl D. Hansing, for encouragement and guidance during the course of this investigation and in the preparation of this manuscript. Appreciation is also expressed to Dr. Charles L. Kramer and Dr. Donald L. Stuteville, the members of the supervisory committee, for their valuable suggestions and for critical review of the manuscript.

Appreciation is extended to Dr. Lewis E. Browder for assistance in greenhouse facilities. Thanks are also due to Dr. Mohammad Aslam for help during various operations.

To the Head of the Department, Dr. John F. Schafer, members of his staff and secretaries, I wish to express my appreciation for the spirit of friendliness and willingness with which they all availed themselves of their services and facilities at times.

Sincere appreciation and thanks are extended to my brother, Inayatullah Jan in Nowshera Cantt, Pakistan, for his sacrifice and constant encouragement during my studies in the United States.

LITERATURE CITED

1. Chatrath, M. S., B. L. Renfro, Y. L. Nene, R. K. Grover, M. K. Roy, D. V. Singh and S. M. Gandhi. 1969. Control of loose smut of wheat with systemic fungicides. Indian Phytopathol. 22:184-187.

2. Crosier, W. F. 1968. Effect of seed and soil applied chemicals upon disease and seedling growth of small grains. Seed and Soil Treatment Newsletter 10:70-74.

3. Demirlicakmak, A., M. L. Kaufmann, and L. P. V. Johnson. 1963. The influence of seed size and seedling rate on yield and yield components of barley. Can. J. Pl. Sci. 43:330-337.

4. Edgington, L. V., and G. L. Barron. 1967. Fungitoxic spectrum of oxathiin compounds. Phytopathology 57:1256-1257.

5. Edgington, L. V., G. S. Walton, and P. M. Miller. 1966. Fungicide selective for Basidiomycetes. Science 153:307-308.

6. Edgington, L. V. 1968. Chemotherapy of loose smut of barley in Canada, p. 20-21. In Moseman, J. G., Fungicidal Control of Smut Diseases of Cereals. U. S. Dept. Agr., Agr. Res. Serv., Crops Res. Div. CR 42-68.

7. Fezer, K. D. 1962. Differential incidence of loose smut among seed-size classes within barley seedlots. Crop Sci. 2:162-164.

8. Fischer, G. W., and G. S. Holton. 1957. Biology and control of smut fungi. The Ronald Press Company, New York, N. Y. 622 p.

9. Freeman, E. M., and E. C. Stakman. 1911. The smuts of grain crops. Agr. Exp. Sta. Bull., Univ. of Minnesota 122:35-45.

10. Hansing, E. D. 1955. Diseases of wheat in Kansas. Loose smut, p. 14-15. In Kans. Agr. Expt. Sta. Bull. 368.

11. _____. 1967. Wheat seed treatment for control of loose smut. Seed and Soil Treatment Newsletter 9:71.

12. _____. 1968. A systemic oxathiin fungicide to control loose smuts, covered smuts, seed rots, and seedling blights of wheat, barley, oats and sorghum, p. 11-15. In Moseman, J. G., Fungicidal Control of Smut Diseases of Cereals. U. S. Dept. Agr., Agr. Res. Serv., Crops Res. Div. CR 42-68.

13. _____. 1967. Systemic oxathiin fungicide for control of loose smut (Ustilago tritici) of winter wheat. Phytopathology 57:814.

14. Hansing, E. D., P. S. Rao, and J. C. Reyes. 1968. Wheat, barley, oat and sorghum seed treatment. Soil and Seed Treatment Newsletter 10:68.

15. Jones, J. P., and R. D. Barnett. 1968. Control of loose smut of wheat and barley in Arkansas with fungicides, p. 3. In Moseman, J. G., Fungicidal Control of Smut Diseases of Cereals. U. S. Dept. Agr. Res. Serv., Crops Res. Div. CR 42-68.

16. Jones, J. P., F. C. Collins, and E. L. Clement. 1969. Control of loose smut of wheat with systemic fungicides. Ark. Farm Res. 18:9.

17. Kaufmann, M. L., and A. D. McFadden. 1960. The competition interaction between barley plants grown from large and small seeds. Can. J. Pl. Sci. 40:623-629.

18. _____ and _____. 1963. The influence of seed size on results of barley yield trials. Can. J. Pl. Sci. 43:51-58.

19. Kiesling, R. L. 1967. Chemical control of loose smut of wheat and barley. The North Dakota Seed Journal 36:3.

20. Kiesselbach, T. A. 1924. Relation of seed size to yield of small grain crops. J. Am. Soc. Agron. 16:670-682.

21. Kilpatrick, R. A., and O. G. Merkle. 1968. Effect of two systemic fungicides on control of loose smut in wheat, p. 29-30. In Moseman, J. G., Fungicidal Control of Smut Diseases of Cereals. U. S. Dept. Agr., Agr. Res. Serv., Crops Res. Div. CR 42-68.

22. Klushnikova, E. S. 1928. Distribution of the mycelium of U. tritici in the tissue of the host plant and anatomical changes caused by it in the structure of the host tissue. Morbi. Plantarum, Leim. 17:125 (Abstr. Rev. Appl. Mycol. 18:712-713, 1928).

23. Krull, Charles F., and Gabriel Robayo, Luis A. Valbuena, Gabriel Rico, Luis E. Castiblanco, and Luis E. Bravo. 1966. Influence of seed size on the incidence of loose smut in Funza barley. Plant Disease Reptr. 50:101-103.

24. Kuiper, J. 1968. Field evaluation of a 1,4-oxathiin for control of cereal smut. Aust. J. Agr. Anim. Hub. 8:756-761.

25. McFadden, A. D., M. L. Kaufmann, R. C. Russell, and L. E. Tyner. 1960. Association between seed size and the influence of loose smut in barley. Can. J. Plant Sci. 40:611-615.

26. Macer, R. C. F. 1968. Preliminary observations on the treatment of wheat and barley with a fungicide to control loose smut in England, p. 23-25. In Moseman, J. G., Fungicidal Control of Smut Diseases of Cereals. U. S. Dept. Agr., Agr. Res. Serv., Crops Res. Div. CR 42-68.

27. Mathre, D. E. 1968. Uptake and binding of oxathiin systemic fungicides by resistant and sensitive fungi. Phytopathology 58:1464-1469.

28. Mathre, D. E. 1970. Mode of action of oxathiin systemic fungicides. I. Effect of carboxin and oxycarboxin on the general metabolism of several Basidiomycetes. Phytopathology 60:671-676.

29. Maude, R. B., and C. G. Shuring. 1968. Preliminary studies on the use of new seed treatments for the control of loose smut of barley. Pl. Path. 17:155-157.

30. Maude, R. B., and C. G. Shuring. 1969. Seed treatment with Vitavax for control of loose smut of wheat and barley. Ann. Appl. Biol. 64:259-263.

31. Moore, M. B. 1936. A method of inoculating wheat and barley with loose smuts. Phytopathology 26:397-400.

32. Morton, D. J. 1961. Trypan blue and boiling lactophenol for staining and clearing barley tissue infected with Ustilago nuda. Phytopathology 51:27-29.

33. Moseman, J. G. 1968. Control of U. nuda with a 1-4 oxathiin fungicide, p. 30-32. In Moseman, J. G., 1968. Fungicidal Control of Smut Diseases of Cereals. U. S. Dept. Agr., Agr. Res. Serv., Crops Res. Div. CR 42-68.

34. Popp, W. 1951. Infection in seeds and seedlings of wheat and barley in relation to development of loose smut. Phytopathology 41:261-275.

35. _____. 1958. An improved method of detecting loose-smut mycelium in whole embryos of wheat and barley. Phytopathology 48:641-643.

36. Russell, R. C. 1950. The whole embryo method of testing barley for loose smut as a routine test. Sci. Agr. 30:361-366.

37. Russell, R. C., and W. Popp. 1951. The embryo test as a method for forecasting loose-smut infection in barley. Sci. Agr. 31:559-565.

38. Ruttle, M. C. 1934. Studies on barley and on loose smut of wheat. Agr. Exp. Sta. Tech. Bull. Geneva, N. Y.

39. Satoris, G. B. 1924. Studies in the life history and physiology of certain smut. Amer. J. Bot. 11:617-647.

40. Semeniuk, Wm, and J. G. Ross. 1942. Relation of loose smut to yield of barley. Can. J. Res. 20:491-500.

41. Simmonds, P. M. 1946. Detection of the loose smut fungi in embryos of barley and wheat. Sci. Agr. 26:51-58.

42. Skvorzoff, S. S. 1937. (Russian title).
A simple method of detecting hyphae of loose smut in wheat grain. Pl. Prot. Leningr. 15, 90-91. Rev. Appl. Mycol. 17:382.

43. Snel, M., B. von Schmeling, and L. V. Edgington. 1970. Fungitoxicity and structure-activity relationship of some oxathiin and thiazole derivatives. Phytopathology 60:1164-1169.

44. von Schmeling, B., and M. Kulka. 1966. Systemic fungicidal activity of 1,4-oxathiin derivatives. Science 152:659-660.

45. _____. 1968. The systemic fungicide 2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin, p. 1-2. In Moseman, J. G., Fungicidal Control of Smut Diseases of Cereals, U. S. Dept. Agr., Agr. Res. Serv., Crops Res. Div. CR 42-68.

46. Waldron, L. R. 1941. Analysis of yield on hard red spring wheat grown from seed of different weight and origin. J. Agr. Res. 8:445-460.

EFFECT OF CARBOXIN ON LOOSE SMUT, *USTILAGO TRITICI*, OF WHEAT

by

ANWAR BAIG

B. Sc., University of Peshawar, Peshawar, Pakistan, 1965
M. Sc., University of Peshawar, Peshawar, Pakistan, 1967

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Plant Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1972

Carboxin applied to Bison wheat seed at the rate of 3 oz per 100 lb. of seed controlled loose smut. The chemical appeared to be absorbed by the germinating seed and translocated to the site of the pathogen during the early stages of seedling development.

The germination rate of seed and per cent of smut infection in different seedling groups were found to be similar whether the seed was treated or not. However, an inverse relationship between coleoptile length and per cent loose smut was apparent.

Small seed were found to have a higher percentage of embryos infected with Ustilago tritici.

Per cent seedling emergence was slightly higher from treated seed than in untreated seed.

The weight of the individual seed did not appear to have any effect on the incidence of loose smut.

Growth of U. tritici mycelia was inhibited in carboxin, but after washing the mycelia with sterilized distilled water, it resumed normal growth. This demonstrated that carboxin was fungistatic rather than fungicidal.