Application Methods Influencing the Effectiveness of Carboxin for Control of Common Bunt Caused by *Tilletia tritici* and *T. laevis* in Spring Wheat

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ABSTRACT

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The concentration of carboxin was determined on individual seeds of seed lots of the bunt-susceptible spring wheat cultivar Laura obtained from different seed treatment operators, farmers, and the manufacturer. These seeds were subsequently infested with a composite of teliospores of virulent races of *Tilletia tritici* and *T. laevis* and seeded in irrigated and dryland sites in 1989 and 1990. At mean bunt infection levels of 19.2 and 50.0%, which occurred in 1990, failure to achieve target rates of carboxin of 550-690 mg/kg in seed appeared to be the greatest cause of poor efficacy. At low mean disease incidence in the nontreated check (7.5% in 1989), or when the mean carboxin concentration exceeded 466 mg/kg of seed, the mean percent control, averaged over seed lots, was high (>90%). At mean carboxin concentrations below 446 mg/kg in 1990, however, control averaged only 63.8%. There was no relationship between mean percent control and mean carboxin concentration at carboxin rates below 446 mg/kg in 1990 or between variability in mean percent control and variability in mean carboxin rates. Failure to achieve target rates and random variability within the seed microenvironment are important factors affecting the efficacy of carboxin at higher disease incidence levels and may explain the lack of consistent control of common bunt with this fungicide.

Additional keyword: Vitavax

Prior to 1950, common bunt incited by *Tilletia tritici* (Bjerk.) G. Wint. in Rabenh. (*Tilletia caries* (DC.) Tul. & C. Tul.) and *T. laevis* Kühn in Rabenh. (*T. foetida* (Wallr.) Liro.) caused extensive losses in spring and winter wheats (*Triticum aestivum* L. and *T. durum* Desf.) in western Canada (4). The disease is initiated by spores of the pathogens on the seed or in the soil that germinate and infect the developing seedling. The fungi develop systemically in the plant and produce bunt balls containing spore masses that eventually replace the kernel (4,10). In addition to the direct loss of grain yields, concentrations of teliospores as low as 0.05% bunt balls (w/w) in harvested grain reduce grain quality (12). In recent years, chemical seed treatments, disease-resistant cultivars, and cultural control practices have nearly eliminated losses due to common bunt.

The highly susceptible nature of some newly registered spring wheats (6) and the western Canada winter wheats has necessitated an increased reliance on seed-treatment fungicides for control of common bunt. Currently, seed-treatment fungicides containing carboxin are extensively used for control of cereal smuts and bunt in North America. Although liquid formulations of carboxin fungicides have been effective elsewhere in controlling common bunt (3,9,11,14), they have not proved to be totally effective in either spring or winter wheat under all environmental conditions (8) and they have provided poor control of soilborne bunt (8,11).

The objective of this study was to evaluate the concentration of carboxin in seed lots obtained from commercial seed treatment operators and on-farm applicators and to relate the levels of carboxin among and within seed samples to their effectiveness in controlling common bunt in spring wheat.

MATERIALS AND METHODS

Carboxin-treated seed of the highly susceptible hard red spring wheat cultivar Laura was received from commercial seed-treatment operators, farmers in southern Alberta, and the manufacturer of carboxin (Vitavax, Uniroyal Chemical Company) and stored dry at 4 C until use. The seed was treated at the sources during the winter of 1988 and the spring of 1989 with a single liquid formulation of Vitavax containing 23% carboxin. The seed treatment source, type of seed treatment equipment, and target rate of carboxin according to label recommendations are listed in Table 1.

The concentration of carboxin on a sample consisting of 38 wheat kernels from each seed lot was determined using acetonitrile extraction followed by gas chromatography; this procedure recovers 95.5 ± 1.3% of carboxin (Uniroyal Chemical Company, *unpublished*). The percent germination (i.e., coleoptile longer than the seed) was also determined for each seed lot by incubating seed on moistened filter paper for 1 wk at 20 C.

A field study to assess the efficacy of carboxin for control of common bunt was conducted in 1989 and 1990. Each year, 1-2 wk before seeding, a portion of the carboxin-treated seed from the seed lots (Table 1) was infested with a mixture of virulent races of *T. tritici* and *T. laevis* at a rate of 0.83 g of air-dried bunt spores per 100 g of seed (8) and stored at 4 C until used. Seed was sown 3 cm deep at a rate of 10 g per 5 m of a single row in a randomized block design with four replications. Treatments were sown at the Lethbridge Research Station (Dark Brown Chernozem, fine loam) on a dryland site on 11 May 1989, on an adjacent dryland site on 2 May 1990, and on an irrigated site on 23 April 1990. At maturity, 200 tillers were harvested from each plot and the number of bunted spikes counted. Control plots were seeded with nontreated seed inoculated with bunt spores or with fungicide-treated seed without bunt spores.

Percent control for each treatment within locations for both 1989 and 1990 was determined using the formula: 100 × [1−(% bunt in the inoculated, carboxin-treated seed lots/% bunt in the inoculated nontreated controls)]. Means, standard errors, and coefficients of variation (CVs) were calculated for the percent control and carboxin concentration on individual kernels. Individual seeds with excessively high concentrations of carboxin (less than 3% of the total seeds tested) were considered outliers and were excluded from the analyses. Scatter diagrams were generated for the CVs for percent control and carboxin concentration. A review of the data showed that the standard errors of the mean percent control generally decreased as the mean increased, but some large means also showed large standard errors. Because no transformation could be used to make variances for the treatments homogeneous and because statistical differences between treatment means were deemed of minor importance, no analysis of variance was conducted to test for significant differences between treatment means.

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Correlation statistics between mean percent control and carboxin concentration for each seed lot and between the corresponding covariance values were determined.

RESULTS AND DISCUSSION

The target rate of carboxin was often not achieved among seed lots sampled in western Canada and, in most instances, the observed rate was less than the target rate (Table 1). Currently, the manufacturer recommends target rates of 550–690 mg/kg of carboxin for control of common bunt in western Canada. The commercial batch drum and Gustafson S-1000 systems delivered carboxin rates lower than the target rates, whereas the Gustafson LBT mister and needle systems delivered rates closer to target rates. For seed lots treated on-farm using a dripper/auger or mister/auger system (Table 1), the observed carboxin rates were higher than the target rate in two of the three seed lots tested. Carboxin distribution among the 38 seeds within seed lots also was highly variable, as evidenced by the magnitude and variability of the standard error values (Table 1). The amount of variation did not appear to be directly attributable to the different types of seed treatment equipment used. For example, target rates and treatment equipment were similar in the 2-SCP and 4-SCP seed lots but the standard errors of the observed rates differed (Table 1).

The mister/auger and dripper/auger systems are designed for on-farm use and are difficult to calibrate accurately. Therefore, farmers might apply the fungicide at rates higher or lower than recommended on the label. The Gustafson S-1000 is a continuous-flow system and the batch mixer is a noncontinuous-flow system. Both, however, can be calibrated readily, as the seed is weighed before treatment and the fungicide amount is regulated. Because the application of carboxin to seeds is automated for the batch mixer and Gustafson S-1000 systems, it is likely that much of the variability in mean carboxin concentration within seed lots is attributable to miscalibration or operation of the systems near or above peak capacity. The Gustafson LBT mister and needle systems are designed to treat small samples (0.5–2.0 kg of seed) for research purposes and should be the most accurate. With all seed fungicide-application systems, reducing the seed lot size or increasing the duration of the tumbling action will allow thorough mixing of seed and fungicide, thereby enhancing the uniformity of fungicide distribution on the seed.

The seed quality in all lots was high, and there was no evidence of phytotoxic response to carboxin treatment in any of the seed lots. Germination and emergence rates in the carboxin-treated uninoculated seed was high (>90%) and was not significantly different from those in the nontreated uninoculated controls.

The mean bunt incidence in the inoculated nontreated controls for the dryland site in 1989 was only 7.5%. The mean level of control was high across all seed lots, ranging from 91.1 to 98.7% (Table 1). In 1990, mean bunt incidence in the inoculated nontreated controls was 19.2 and 50.0% in dryland and irrigated sites, respectively (Table 1). The higher bunt incidence in 1990 was probably attributable to earlier seeding dates than in 1989. Seeding into cool soils increases the incidence of the disease because bunt fungi have relatively low optimal temperatures for spore germination and infection (1,8). The difference in disease incidence between the two locations in 1990 may be attributed to the higher available moisture in the irrigated site. Wet soils, which warm more gradually in the spring, remain conducive to bunt infection for a longer period than dry soils (15).

The mean control among seed lots in 1990 ranged from 44.4 to 97.7% across locations and was similar for both locations despite a 2.5 times higher bunt level in the irrigated site than in the dryland site (Table 1). The incidence of bunt did not appear to directly affect the efficacy of carboxin, except under conditions promoting low bunt severity in 1989 when percent control improved.

At mean carboxin rates at or exceeding 466 mg/kg, bunt control was high, averaging 92.2 and 90.2% for the dryland and irrigated sites, respectively, in 1990. However, variability was apparent in the level of control and the magnitude of the standard error values between seed lots and locations (Table 1). For seed lots 7-SG and 11-U, lower levels of control than for other seed lots with >466 mg/kg of carboxin were observed at the dryland and irrigated sites. Complete control of common bunt was also not observed for seed lot 12-SG, which had the highest mean carboxin level.

At carboxin rates below 446 mg/kg, bunt control was lower, averaging 61.8 and 65.8% in 1990 for the dryland and irrigated sites, respectively. Correlation between mean percent control and mean carboxin concentrations in seed lots with concentrations below 446 mg/kg was nonsignificant ($P = 0.73$). Differences in percent control among treatments were apparent even when mean concentrations of carboxin were similar (Table 1). For example, seed lots 2-SCP, 3-E, and 5-SG had similar mean carboxin rates but varied in percent control. Although the mean percent control of the lowest carboxin treatment rate, seed lot 8-U, ranked among the lowest for percent control (Table 1), it was similar to seed lots such as 2-SCP and 5-SG with mean rates approximately 2.5 times higher.

Table 1. Carboxin concentration on seed of spring wheat cultivar Laura sampled from different sources after treatment with carboxin at different application rates and percent control of common bunt in the field during 1989 and 1990

Seed lot	Source[a]	Treatment equipment	Target rate (mg/kg)[b]	Carboxin concentration (mg/kg)[c]	Percent control 1989 Dryland	Percent control 1990 Dryland	Percent control 1990 Irrigated
8-U	RS	Gustafson LBT mister	110	140.8 ± 11.9[d]	91.1 ± 10.4	56.1 ± 6.8	47.5 ± 4.6
10-U	RS	Gustafson LBT mister	280	242.4 ± 15.5	98.7 ± 1.3	71.8 ± 6.3	77.6 ± 2.6
4-SCP	CM	Gustafson S-1000	550–690	259.6 ± 26.1	93.9 ± 2.2	65.8 ± 9.1	69.4 ± 5.8
5-SG	CM	Batch mixer	550–690	326.2 ± 29.8	96.4 ± 2.1	44.4 ± 15.5	69.6 ± 6.0
2-SCP	CM	Gustafson S-1000	550–690	345.1 ± 39.3	91.1 ± 5.1	59.0 ± 11.8	56.0 ± 5.9
3-E	CM	Batch mixer	550–690	375.1 ± 27.9	95.8 ± 4.2	82.9 ± 5.1	72.4 ± 4.1
6-SG	OF	Mister/auger	550–690	446.7 ± 40.6	92.7 ± 4.5	52.6 ± 14.7	68.1 ± 1.1
11-U	RS	Gustafson LBT needle	550	466.6 ± 39.0	96.5 ± 2.0	94.7 ± 3.8	82.3 ± 2.6
9-U	RS	Gustafson LBT mister	550	671.4 ± 57.1	95.8 ± 4.2	94.2 ± 4.6	95.1 ± 1.6
7-SG	OF	Mister/auger	550–690	697.0 ± 79.8	94.4 ± 1.9	84.3 ± 10.1	92.1 ± 0.7
12-SG	OF	Dripper/auger	550–690	1,085 ± 126.7	96.4 ± 2.1	97.7 ± 0.8	92.9 ± 1.7
Mean[e]					94.8 ± 0.9	73.1 ± 3.6	74.7 ± 2.4

[a] RS = research (Uniroyal Chemical Company), CM = commercial, OF = on-farm.

[b] Desired concentration of carboxin on seed as specified on the label or by the manufactuer for experimental purposes.

[c] Mean concentration measured per seed on 38 seeds.

[d] Mean ± standard error.

[e] Mean percent bunt ± standard error for inoculated nontreated controls were 7.5 ± 7.9 for 1989 dryland study, 19.2 ± 3.1 for 1990 dryland study, and 50.0 ± 3.7 for 1990 irrigated study.

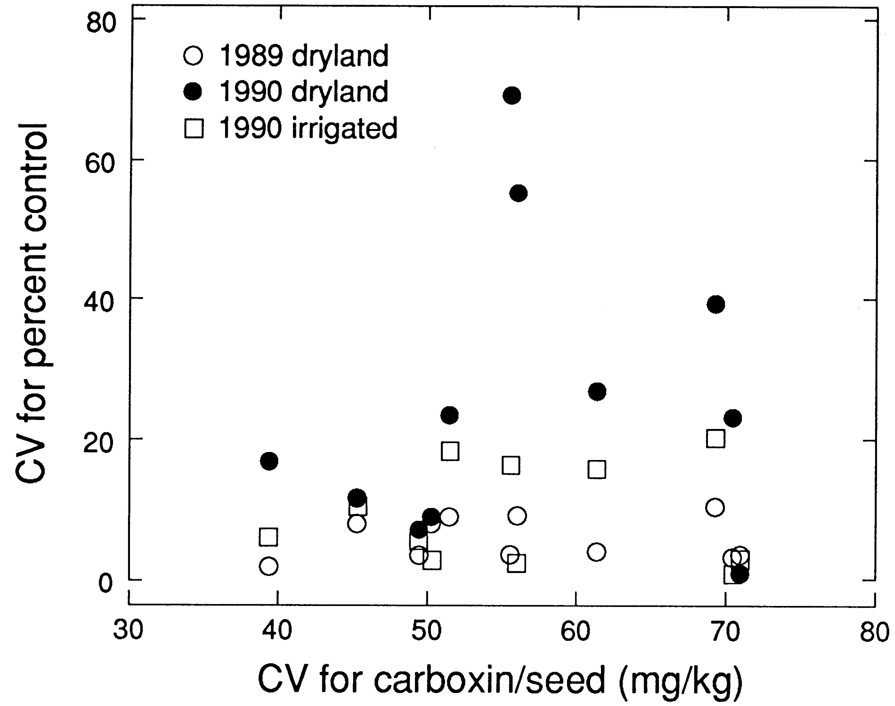

Fig. 1. Coefficients of variability (CVs) for concentration of carboxin on the seed vs. CVs for percent control of common bunt in the field during 1989 and 1990 at Lethbridge, Alberta, Canada.

This suggested that there was no relationship between mean carboxin concentration on the seed and percent control when the mean carboxin rates were below 446 mg/kg. Furthermore, correlation statistics showed that there was no relationship ($r^2 = 0.07$, $P = 0.73$) between the CVs of carboxin concentration and the CVs of percent control for all seed lots evaluated, regardless of the observed carboxin concentration (Fig. 1). This suggested that variability in mean concentration of carboxin on individual seeds was not associated with variability in percent control.

Failure to observe a dose-response relationship between carboxin and percent control at rates below 446 mg/kg suggested that carboxin efficacy for control of common bunt was highly variable and unreliable at suboptimal concentrations on the seed. However, the lack of a relationship between covariances of percent control and those of mean carboxin concentration implied that the variability in the fungicide's efficacy was not attributable to the proportion of seeds with low levels of fungicide. It is likely that poor efficacy at suboptimal carboxin concentrations is attributable to effects of random variability within the seed microenvironment. Some factors that may contribute to random environmental variability and may act at the level of the developing seedling include soil moisture, temperature, pH, host resistance, and pathogen aggressiveness (7).

It was not possible in this study to determine whether carboxin was distributed unevenly on the surface of individual seeds. Uneven distribution over the seed surface also may contribute to variability in percent control. Also, it was not possible to control the storage conditions of treated seed before receipt. Poor storage conditions could contribute to some of the variability in percent control among seed lots possessing similar mean carboxin concentrations. The activity of carboxin is highly stable when treated seed is stored under cool, dry conditions (Uniroyal Chemical Company, *unpublished*).

The optimum concentration of carboxin for control of common bunt in spring wheat is unknown. It is unlikely, however, that a single optimum rate exists for bunt control by this fungicide in all environments, since the efficacy of carboxin-based seed-treatment fungicides varies among locations and years (8). Carboxin applied to the seed is absorbed primarily through the wheat caryopsis (2). For example, in water-saturated soils, some of the carboxin could diffuse away from the seed and become unavailable for absorption. The variable rate of oxidation of carboxin to sulfoxide (5), a less fungitoxic compound (13), also may contribute to the variable activity of carboxin in controlling common bunt.

In summary, the variable efficacy of carboxin for controlling common bunt in spring wheat under the conditions of this study was attributed to: 1) failure to attain rates recommended on the label and 2) random variability of seed microenvironment. The methods used in this study—early planting of heavily infested seed into cold soils and infestation of the seed with teliospores of common bunt after rather than before treatment with carboxin—probably would not be encountered in a commercial operation. Under conditions of the normally low disease severity encountered in the field, carboxin likely will continue to give good control of common bunt in spring wheat, provided that the mean fungicide level within the seed lot is within the range recommended on the label. However, recent registration of spring wheats highly susceptible to common bunt (6,7), coupled with the very low bunt incidence required to cause downgrading of wheat, illustrates the need for seed-treatment fungicides that are efficacious on highly susceptible cultivars over a wide range of environmental conditions.

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