FINAL REPORT

Blackleg control through enhanced straw decomposition

P. V. Blenis

P. S. Chow

G. R. Stringam

Department of Agriculture Food and Nutritional Science

University of Alberta

Edmonton, Alberta

Abstract

Blackleg is an extremely important disease of canola and all current control measures have some drawbacks. Because the blackleg fungus survives only in plant residues, it is possible that the incidence of this disease could be reduced if the rate of straw decomposition could be increased. This study evaluated the potential for using selected cultivars, chemical amendments and decay fungi to enhance straw decomposition. In two separate years, the roots and stems of 36 cultivars of *Brassica napus* or *B. rapa* were buried or left on the soil surface. There were no significant differences in stem decomposition among cultivars within species, whereas burial increased stem decomposition and roots underwent less decomposition than stems. None of three tested chemicals increased straw breakdown in a greenhouse test. Fifty five different fungal isolates were compared on the basis of their tolerance of low water potential and cool temperature, their inherent ability to colonise and decompose sterilised and unsterilised straw and their ability to replace *L. maculans* from blackleg-infested straw. Although none of the fungi were particularly effective in decomposing unsterilised straw or replacing the pathogen from infested tissues, the genera *Cyathus* and *Coprinus* appeared to have the greatest potential as biological control agents.

Background and objectives

The blackleg fungus exists in avirulent and virulent forms. The virulent form was first found in Saskatchewan in 1975 and was reported from Alberta in 1983. This virulent form is very destructive and has caused yield losses of up to 56% in individual fields. The common variety Westar is highly susceptible to the disease and although the variety Quantum is resistant to blackleg, most varieties are still either moderately susceptible or moderately resistant. It has been stated that "there is a very real threat of blackleg continuing to cause devastating crop losses in Canada if susceptible rapeseed cultivars continue to be licensed and widely grown".

All current or proposed control measures have some drawbacks. Crop rotation can be effective but some growers are reluctant to wait four years before planting a second crop. Deep tilling may help control the disease but is contrary to a trend towards reduced or minimum tillage. Resistant varieties have been used to control many diseases. However, development of resistance is time consuming and resistant varieties may "break down", if new virulent races increase in number. This difficulty is especially pronounced if resistance is used as the sole control measure.

The blackleg fungus survives only in plant residues. Thus the disease should be reduced by increasing the rate of straw decomposition. Enhanced residue breakdown may also reduce the need for deep tillage, extend the durability of resistant cultivars, and increase soil nitrogen.

There are a number of different ways in which the rate of straw decomposition could be enhanced 1) varieties could be selected for rapid decomposition rates with due concern for lodging resistance, 2) chemicals could be applied to straw to increase the rate of breakdown, or 3) wood decay fungi, which have a remarkable ability to decompose lignified substrates, could be applied to destroy the canola straw. The overall objectives of this project were to evaluate the potential of those three methods for increasing the rate of straw decomposition. In evaluating the potential of wood decay fungi to serve as biological control agents, consideration was given to their tolerance of low water potential and temperatures, their inherent ability to colonise and decompose sterilised and unsterilised straw and their ability to replace *L. maculans* from blackleg-infested straw.

Experimental method

Evaluation of differences among cultivars in straw decomposition. Experimental material was obtained from canola regional variety trials established at Ellerslie, Alberta. Cultivars were planted May 4 in four-row plots, which were 6 m long with 23 cm between rows and approximately 2 cm between plants within rows. *Brassica rapa* and *B. napus* were harvested on August 20 and August 30, respectively. On September 1, 1994,

approximately 20 pieces of stubble, including the roots and about 10 cm of stem, from 23 cultivars of *B. napus* and 13 cultivars of *B. rapa*, were collected at random from the center rows, and air dried. From the original collection, two pieces, in each of three diameter size classes, were selected. They were cut into separate 5 cm stem and root pieces at the point of cotyledon attachment, dried for 4 days at 60 C, and weighed. On October 9, the straws were put into 10 cm X 10 cm nylon packets with a mesh size of 1.5 mm and placed in the field. The field design was a split-split-split plot. There were three blocks, 6 m X 6 m in size, 1.5 m apart, to which the three different size classes of straw (average weights 0.67, 0.5, and 0.40 g) were randomly assigned. Stems and roots were randomly assigned to two 6 m X 3 m subplots. These subplots were further divided into two 3 m X 3 m sub-sub-plots to which the burial treatments were randomly assigned with packets either secured to the soil surface or buried 10 cm in the soil. The 36 cultivars were randomly arranged on a 6 X 6 grid within each sub-sub plot. In August, 1995 the straws were removed, gently washed, dried and weighed. Data were analyzed with SAS PROC MIXED (Littell et al 1996). The experiment was repeated in 1995-1996; of the 36 cultivars tested, 22 had been evaluated in the previous year. The straws were slightly larger, the average weights of the three size classes being 1.1, 0.65, and 0.32 g.

Evaluation of chemical treatments to enhance decomposition. Straws of the cultivar Alto were washed, cut into 5 cm long stem and root portions, oven dried and weighed. A fully randomized design with four treatments was employed. The treatments corresponded to four chemicals as follows: Concentrated sulfuric acid-urea [106.6 mL of concentrated H_2SO_4 and 120.2 g of urea]; Dilute sulfuric acid-urea [20 mL of the preceding solution + 180 mL of water]; Hydrogen peroxide-iron [0.1 M sodium acetate, 0.44 mM $FeSo_4 \bullet 7H_2O$, 1% H_2O_2]; Distilled water. The treatments were randomly assigned to six stems and six roots each. The straws were then dipped in the appropriate solution, placed in 8 cm pots containing field soil, put in a greenhouse at 18 C and watered daily. After 8 weeks the straws were removed, dried and weighed.

Evaluation of antibiotic production. Straws of the cultivar Alto were cut into 5-cm-long root and stem portions, placed in 18 X 150 mm test tubes containing 3 g of Metromix® at 50 % moisture content, and autoclaved. The experimental design was a randomized

complete block with three blocks and 13 treatments. The thirteen treatments consisted of two isolates each of *Bjerkandera adusta*, *Phanerochaete chrysosporium*, *Schizophillum commune*, *Phlebia gigantea*, *Trichoderma longibrachiatum* and *Peniophora rufa* together with an uninoculated control treatment. Cultures of these fungi were maintained on malt agar and 5 mm diameter plugs of mycelium were cut from the plates and used to inoculate the straws. Blocking was achieved by incubating test tubes in the same block in the same tray. Incubation was at room temperature in darkness. After 51 days of incubation, the straws were ground to a fine powder in liquid nitrogen. This powder was extracted with 10 mL of water for 30 minutes, and filtered through Whatman® #1 filter paper. The resultant solution was stored frozen for 7 days in 20 mL scintillation vials prior to evaporation under vacuum. One mL of distilled water was added to each vial and the resultant solution was filter sterilized.

A single isolate of *L. maculans* was grown on V-8®-Rose Bengal agar and used as the test fungus. Three cultures of the fungus were asceptically comminuated in a Waring® blender and 300 µL of solution were spread evenly over petri dishes containing V-8®-Rose Bengal agar to create a fungal lawn.

Whatman® #1 filter paper discs, 18 mm in diameter were saturated with the extract from the canola straws and placed on the fungal lawns. Diameter of the inhibition zone surrounding the saturated discs was measured after 7 days.

Decay fungi tested. In most experiments 55 different fungi were tested (Table 1). In general, these isolates were obtained either from the Canadian Forest Service, the University of Alberta Mold Herbarium, or were collected specifically for this project. Identification of fungi as *Coprinus* or *Cyathus* was occasionally based on cultural morphology rather than on fruiting bodies. The fungi were classified into five groups: *Phanerochaete chrysosporium* (Pha), wood decay fungi (Rot), unknown fungi isolated from canola straw (Unk), *Cyathus* (Cya) and *Coprinus* (Cop).

Tolerance of wood decay fungi to reduced water potential. The 55 different test fungi were grown on malt yeast agar amended with glycerol at molarities of 0, 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 M. Colony diameters for each isolate were measured once the fungus had

grown about half way across the plate without glycerol. Linear interpolation was used to calculate the glycerol concentration at which colony diameter was reduced to 50 % of that occurring on glycerol-free control plates. This value subsequently was expressed as a water potential. The experiment was conducted twice and analyzed as a randomized complete block with the two replicate experiments considered as blocks.

Tolerance of wood decay fungi to reduced temperature. The 55 different test fungi were grown on malt yeast agar and placed in incubators at either 22 or 13 C. Colony diameters were measured when the isolate growing at 22 C had grown about one third of the way across the plate. The ratio of growth at 13 C to growth at 22 C was used as an index of the ability of the fungi to grow at reduced temperature. The experiment was replicated four times; the temperatures assigned to the incubators were interchanged after each replicate. Data were analyzed as a randomized complete block design with the four replicate experiments considered as blocks.

Ability of decay fungi to colonize unsterilised straw. Plastic trays were filled with field soil with a moisture content of 28 %. That particular water content was chosen as a consequence of preliminary testing which determined that a moisture content of 28% would result in a water potential equal to -0.33 bars, the approximate value of field capacity. In each tray, 57 5-cm-long root segments of the variety Alto were half buried vertically in the soil.

Preliminary studies also were done to determine the relative growth rate of the different fungi. With this information it was possible to establish fresh cultures at different times so that the colonies had grown between one half and one third of the way across the petri dish at the time when they were used.

The straws were inoculated by placing 5 mm diameter plugs of mycelium of the 55 test fungi (plus sterile agar as a control) on the tops of the roots. The trays were then sealed and every 4 weeks the trays were reweighed and additional water was added to reestablish the water content. Attempts were made to reisolate the fungi by removing the roots, splitting them in half to expose the internal tissues and asceptically transferring 4 small pieces of tissue to culture media. A randomized complete block design was used with two

blocks each with 10 trays. The average time between inoculation and reisolation was 85 days for the first replicate and 42 days for the second. The cultural morphology of the isolates recovered from the straws was compared with that of the isolates used for inoculation and the percentage of straws yielding the inoculated fungus, *Cyathus* and *Coprinus* was determined.

Ability of decay fungi to decompose sterilised and unsterilised straw. Straws of the cultivar Alto were cut into 5-cm-long root portions, oven dried, weighed, placed in 18 X 150 mm test tubes containing 3 g of Metromix® at 50 % moisture content, and autoclaved. A 5 cm diameter plug of each of the 55 test fungi (plus sterile agar as a control) was placed on each straw. The experimental design was a randomized complete block with three blocks, based on initial straw weight. After 6 weeks the straws were removed, oven dried and reweighed. An experiment using unsterilised straw was conducted in a similar fashion, except that the straws were air dried, rather than oven dried, prior to being placed in the test tubes. Furthermore, an additional 20 straws were air dried, weighed, oven dried and then reweighed to develop a conversion factor that was used to adjust the initial air dry weights to an oven dry weights.

Ability of decay fungi to replace L. maculans from colonized straws. Straws of the cultivar Alto were cut into 5-cm-long root portions, and placed in 18 X 150 mm test tubes containing 3 g of Metromix® at 50 % moisture content, and autoclaved. A total of 280 straws were inoculated with *L. maculans* by placing a 5-cm diameter plug of inoculum on each straw. An additional 56 straws were left uninoculated as controls. After 7 weeks, 5-cm diameter plugs of the 55 test fungi (plus sterile agar as a control) were used to inoculate five *L. maculans*-infested and one control straw each. After 12 more weeks attempts were made to reisolate *L. maculans* onto V-8®-Rose Bengal agar.

Results and discussion

Evaluation of differences among cultivars in straw decomposition. Percentage decomposition, averaged over both experiments, was 38.3 % and 34.1 % for *B rapa* and *B. napus*, respectively (Fig. 1). The average initial weight of the *B. rapa* straw was only about 57 % of that of the *B. napus* straw. Thus, although the combination of lower starting weight, together with greater decomposition caused the final straw weight of *B. rapa* to be little more than half that of *B. napus*, it is clear that the former factor is of much greater importance in reducing final straw weight.

In the first experiment, the stems of *B. rapa* were significantly more decomposed than those of *B. napus*, although there was no difference in root breakdown. In the second experiment, there was a three way interaction between species, straw segment and burial treatment. One perspective of this interaction is that for buried straw, the two species differed in root decomposition only. An alternative perspective is that for *B. rapa*, the burial effect was greater for the root than for the stem portion of the plant. The practical implication of these interactions between species or burial and stem segment is that treatment effects should not be assumed to be consistent for the two plant parts. However, given that the interactions were not great, and given that roots seem to persist longer than stems, it would seem reasonable that roots alone would be a good experimental material.

Variation among cultivars within species was significant in the second, but not the first, experiment. In the second experiment, the estimated standard deviation of the cultivar effect (and 95 % confidence interval of that standard deviation) was 4.5 (2.7 to 5.7) with no significant difference in variance between *B. rapa* and *B. napus*, and no interaction between the cultivar effect and either burial or plant segment effects. The inconsistent results between the two experiments may have been a consequence of different environmental conditions. Although the varieties used in the two experiments were not identical, repeating the data analysis only for varieties used in both 1995 and 1996 gave a similar result: cultivar effects were significant only in the latter experiment. Furthermore,

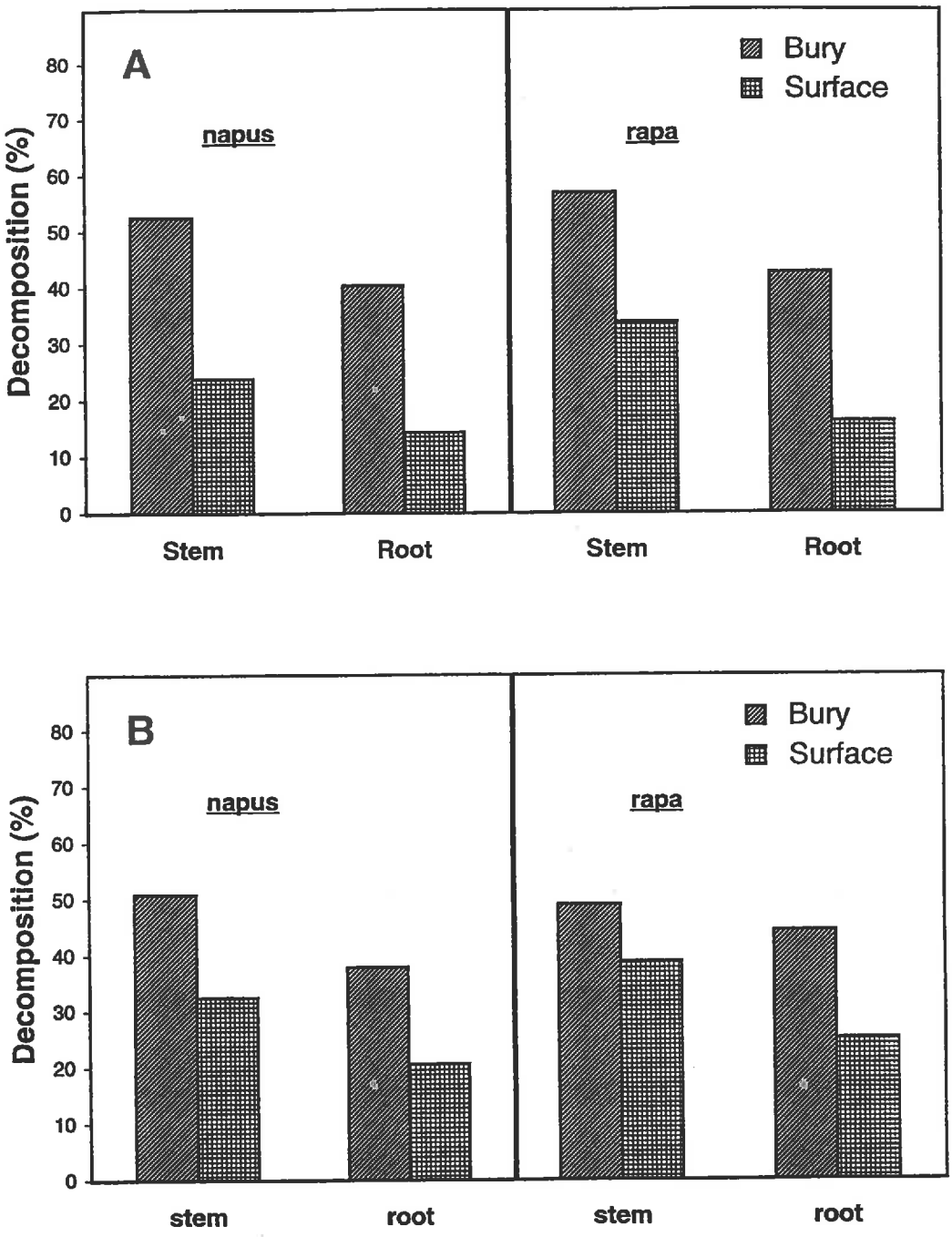

Fig. 1. Effects of burial, plant portion (shoot vs. root) and species (*B. napus* vs. *B rapa*) on straw decomposition in the first (1994-1995 [A]) and second (1995-1996 [B]) experiments. In the first experiment, plant portion, burial, species and plant portion X species effect were significant at 0.0002, 0.0001, 0.0001, and 0.0005, respectively. In the second experiment, location, burial, species and location X burial X species were significant at 0.0073, 0.0001, 0.0593 and 0.0024, respectively.

for individual cultivars, percent decomposition was not consistent between the two experiments, with the estimated correlation coefficient being only 0.15 (P = 0.50). The obvious implication of this inconsistency is that it may be difficult to exploit cultivar differences to change rates of straw decomposition.

On average, roots lost about three quarters and two thirds as much weight as stems in the first and second experiments, respectively. However, as indicated above, there were significant interactions of stem segment with the other treatments. Of all treatments, burial had the greatest effect. In the first and second experiments, buried straw underwent more than twice as much and approximately 1.7 times as much decomposition, respectively, as straw on the surface. Thus the effect of burial on canola straw seems to be of the same order of magnitude as the effect of burial on cereal straw (Summerell and Burgess, 1989; Christensen, 1986). Since the difference in decomposition between buried and unburied straw was 26 and 16 percentage points in the first and second experiments, respectively, and since the standard deviation of the cultivar effect was only 4.5 percentage points in 1996, it would seem that considerable effort would be required to select cultivars to produce a similar sized decomposition effect as burial. Of all cultivars placed on the surface in 1996, Quantum underwent the most rapid decomposition (40 %). However, this was numerically less than the average decomposition of all buried cultivars (45 %).

In summary, these experiments indicated that 1) *B. rapa* straw generally underwent greater decomposition than *B. napus* straw, but differences in straw weights between the species after one year were more due to differences in starting weights than in rates of decomposition. 2) Differences in decomposition rate among cultivars within species were not consistent and when significant were fairly small relative to the large effect of straw burial and 3) Species and burial effects differed slightly between stems and roots, but because roots are the more persistent they may be the best experimental material for studies such as this.

Evaluation of chemical treatments to enhance decomposition. The mean percentage straw weights remaining 8 weeks after treatment were 86%, 85%, 92% and 92 % for the

distilled water, dilute sulfuric acid, concentrated sulfuric acid and hydrogen peroxide-iron treatments. These means were not significantly different (P=0.43). Although "brown rot" fungi, which rapidly reduce the strength of lignified substances (Koenigs 1974), reportedly use a combination of iron and hydrogen peroxide to decompose wood, those chemicals did not enhance straw decay in this study. Similarly, although urea-sulfuric acid accelerated the decomposition of wheat and barley straw (Smith and Jackson, 1987) it also was ineffective in this study. It is possible of course that the particular conditions under which these studies were conducted were not conducive to the use of these chemicals. In particular, daily watering of the pots may have caused the soil to be too wet for effective decay. Although the use of these compounds does not appear to be too promising at this time, further experimentation under a range of soil water conditions would be necessary before their potential could be discounted.

Evaluation of antibiotic production. There was no evidence that *L. maculans* was inhibited by the extracts from straw. As in the preceding experiment, the negative results might have been due to the methods employed. For example, if a different solvent other than water had been used, or if the test fungi had been given more time or less time to colonise the straw or if the extracts had been concentrated further, a different result might have occurred. Nevertheless, the pathogen often grew directly on top of the extract-treated disks and showed absolutely no susceptibility to the treatments. Thus it does not seem likely that the test fungi are likely to be capable of inhibiting the pathogen by water-soluble antibiotics.

Tolerance of wood decay fungi to reduced water potential. Although there were no significant differences among the different groups of fungi in terms of tolerance to low water potential (P=0.15), the wood decay fungi seemed to be more tolerant than the other groups which in turn were approximately equal in their ability to tolerate low water potential (Fig. 2). An effective biological control agent should have the ability to grow under conditions of reduced moisture. According to this criterion, it would appear that decay fungi, as a group, are very well suited.

Tolerance of wood decay fungi to reduced temperature. There were significant differences among the fungal groups in their ability to tolerate reduced temperatures (Fig.

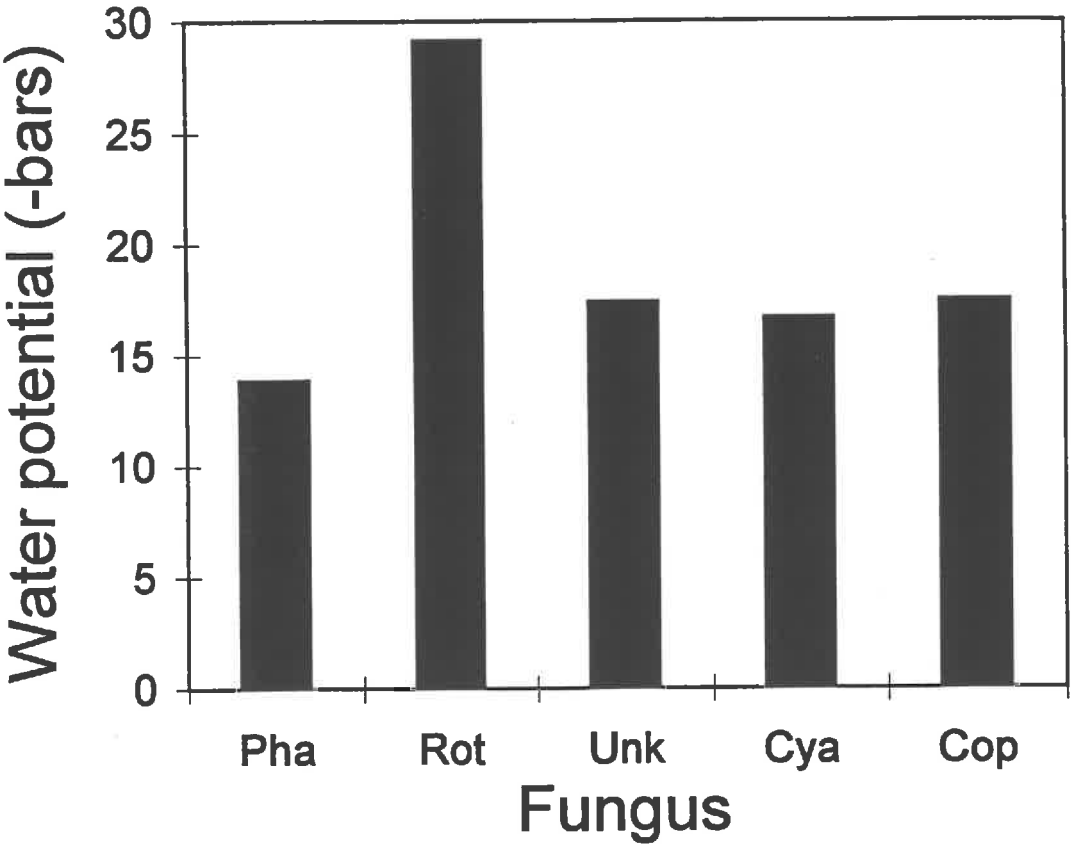

Fig. 2. Water potential at which the growth of five groups of test fungi was reduced to one half that occurring on glycerol amended agar. Differences among the groups were not significant (P=0.15). Pha = *P. chrysosporium*, Rot = fungi isolated from rotting wood, Unk = unknown fungi isolated from canola straw, Cya = *Cyathus*, Cop = *Coprinus*.

3). The group of fungi recovered from canola straw and classified as "unknown" were the most tolerant followed by *Coprinus*, *Cyathus*, the decay fungi and *P. chrysosporium*. All groups were significantly different from each other with the exception of *Cyathus* and *Coprinus* which were not different from each other and *P. chrysosporium* and the decay fungi, which also were not significantly different from each other.

The three groups of fungi recovered from canola straw (*Coprinus*, *Cyathus* and the "unknown" fungi) were relatively better able to tolerate reduced temperatures than those fungi associated with wood decomposition, (the "Rot" fungi *and P. chrysosporium*). The reasons for this are not clear but the practical implication is that judging by the criterion of temperature tolerance, fungi from canola have the best potential for enhancing stem decomposition. The greatly reduced growth of *P. chrysosporium* at the cooler temperature indicates that its usefulness as a biological control agent in this region may be limited.

Ability of decay fungi to colonize unsterilised straw. There was no significant difference in the frequency with which either the inoculated fungi, *Cyathus* or *Coprinus* were reisolated from the straws (Fig. 4). However, the numerical differences in isolation frequencies were quite large and the absence of a significant treatment effect appears to be due to the large experimental error, measured as the replicate by treatment interaction. For example, when *Cyathus* was used to inoculate the straw, it was recovered 0.0 % and 30.0 % of the time from the first and second replicates respectively and when *Coprinus* was used to inoculate the straw, it was recovered 9.3 % and 46.7 % of the time from the first and second replicates respectively. *Cyathus* and *Coprinus* also were reisolated frequently from straws upon which they had not been placed. Recovery of decay fungi from the straws on which they had been placed was quite low.

Although differences among the different groups of fungi were not statistically significant, the results suggest that both *Cyathus* and *Coprinus* may be quite aggressive in colonizing unsterilised straw. The high rates of recovery of these fungi in the first replicate, both from straws on which they had been placed and from nearby straws, indicates their potential for invading unsterilised substrate. The lack of recovery of these two fungi in the

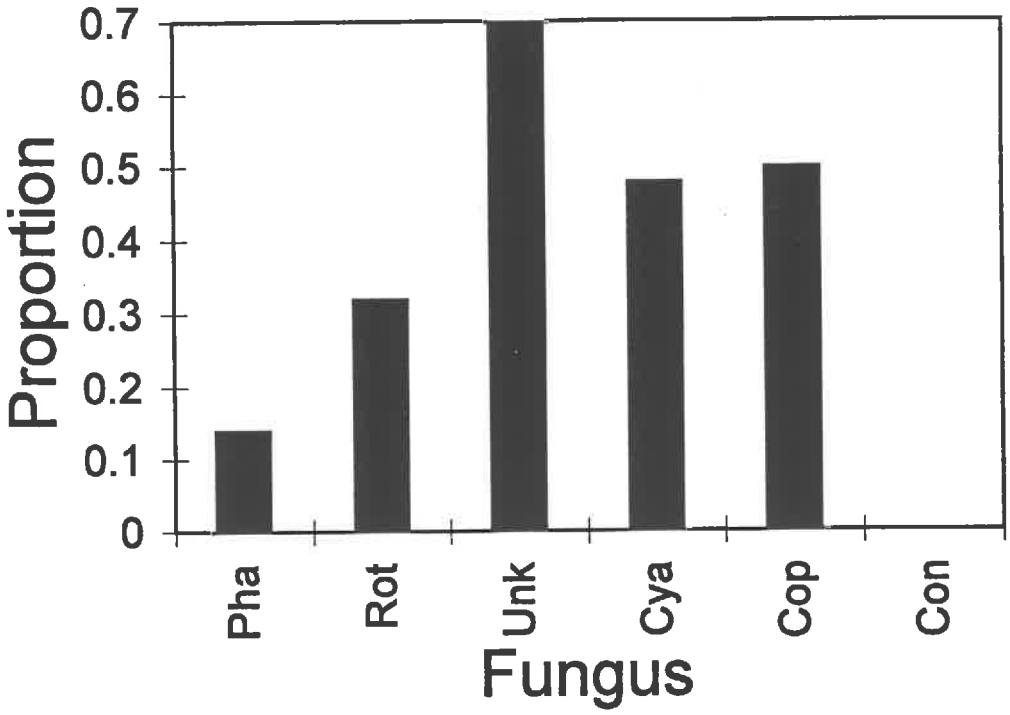

Fig. 3. Diameter growth of five groups of test fungi at 13 C as a proportion of their growth at 22 C. All groups were significantly different except: 1) Cya and Cop were not different, 2) Pha and Rot were not different. Pha = *P. chrysosporium*, Rot = fungi isolated from rotting wood, Unk = unknown fungi isolated from canola straw, Cya = *Cyathus*, Cop = *Coprinus*.

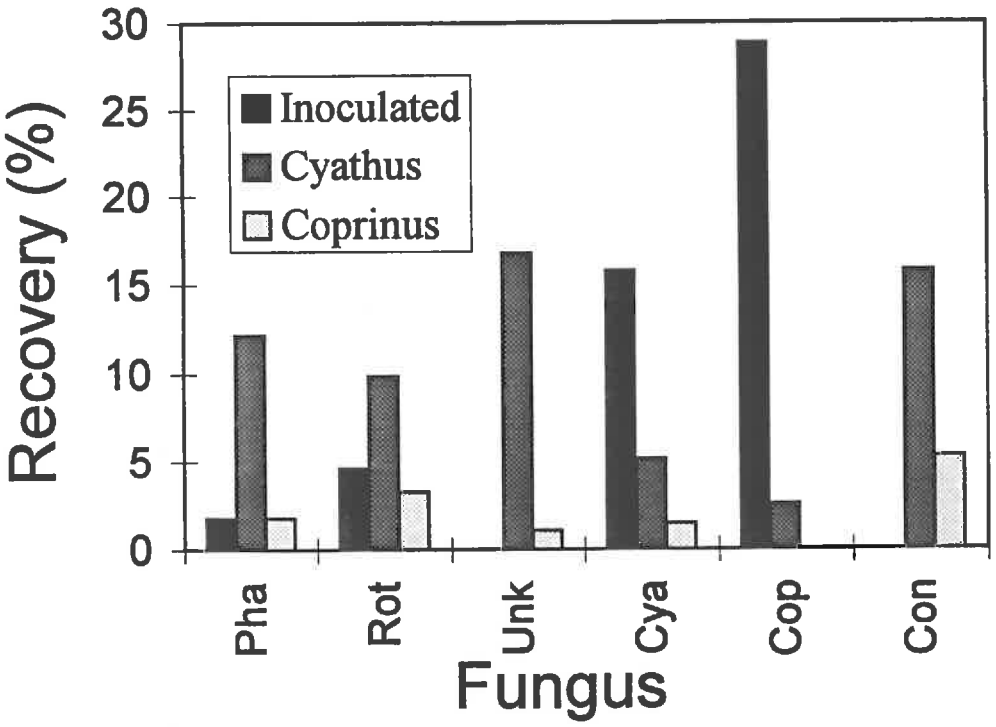

Fig. 4. Percentage of reisolation attempts yielding the inoculated fungus, Cyathus, or Coprinus after non-sterile roots were inoculated with five groups of test fungi. There were no significant differences among the groups of test fungi for any of the reisolated fungi. Pha = P. chrysosporium, Rot = fungi isolated from rotting wood, Unk = unknown fungi isolated from canola straw, Cya = Cyathus, Cop = Coprinus.

second replicate may have been due to the greater length of time before reisolations were attempted. It is possible *Cyathus* and *Coprinus* had previously colonized the straws but been replaced by other fungi.

Ability of decay fungi to decompose sterilised and unsterilised straw. Sterilized straw inoculated with *P. chrysosporium* underwent significantly more decomposition than straws inoculated with any of the other fungi (Fig. 5). All of the other fungal treatments, except for *Coprinus* and the control, resulted in a significant level of decomposition, although they did not differ significantly from each other. All of the unsterilised straw underwent some decomposition and there was no difference between any of the fungal treatments and the control (Fig. 6). The ability of *P. chrysosporium* to thoroughly break down straw at room temperature is not unexpected, given the frequency with which it has been suggested as a biological pulping agent. The relatively low rate of decomposition of unsterilised versus sterilised straw indicates that under field conditions, inoculation with these fungi may not be that effective in increasing the rate of straw decomposition.

Ability of decay fungi to replace L. maculans from colonized straws. The differences among the fungi approached significance (P=0.056) with the lowest recovery of *L. macularis* coming from those straws which were not inoculated with fungi (Fig. 7). These results were quite unexpected and additional work is in progress to test the repeatability of this phenomenon.

Impact

The results of these experiments lead to the following conclusions:

- Differences in rates of straw decomposition among varieties within species were relatively small compared to the effect of burial on straw decomposition. This in turn suggests that attempting to select varieties with increased rates of straw decomposition may not be as practical as attempting to develop cultural methods for enhancing straw decomposition.

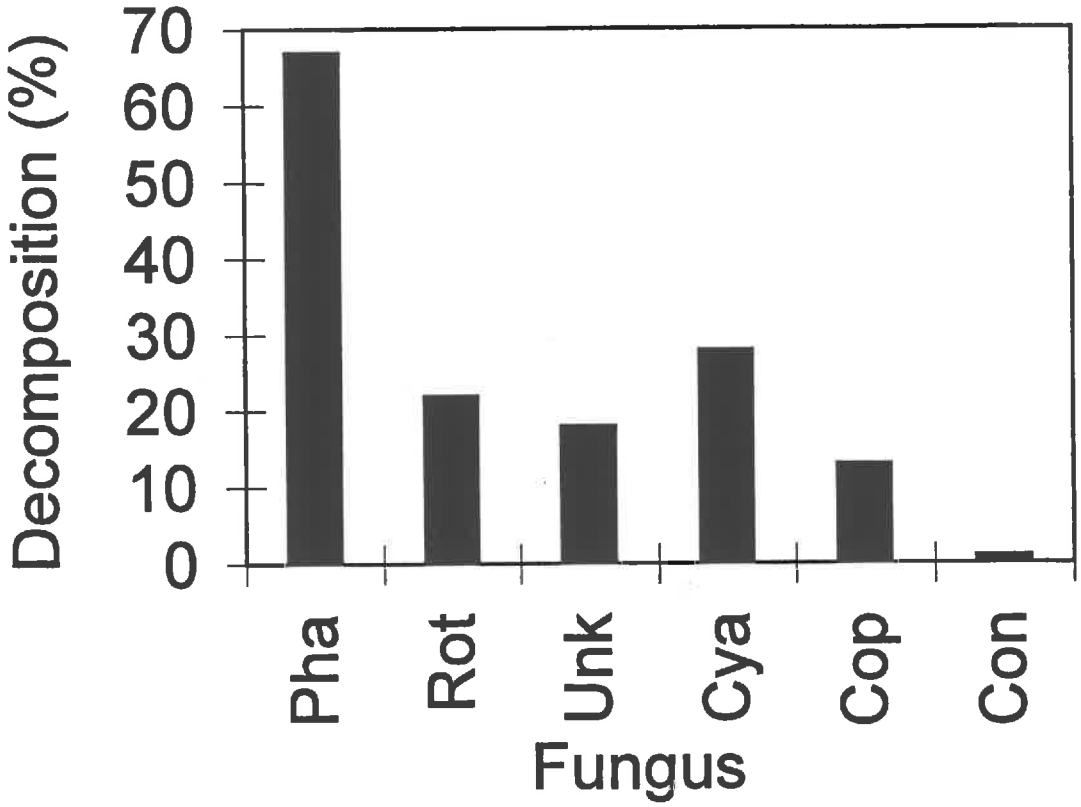

Fig. 5. Percent decomposition of sterilized straw inoculated with five groups of fungi. Decomposition from Pha was greater than that from the other groups. All groups except Con caused significant decomposition. Pha = *P. chrysosporium*, Rot = fungi isolated from rotting wood, Unk = unknown fungi isolated from canola straw, Cya = *Cyathus*, Cop = *Coprinus*.

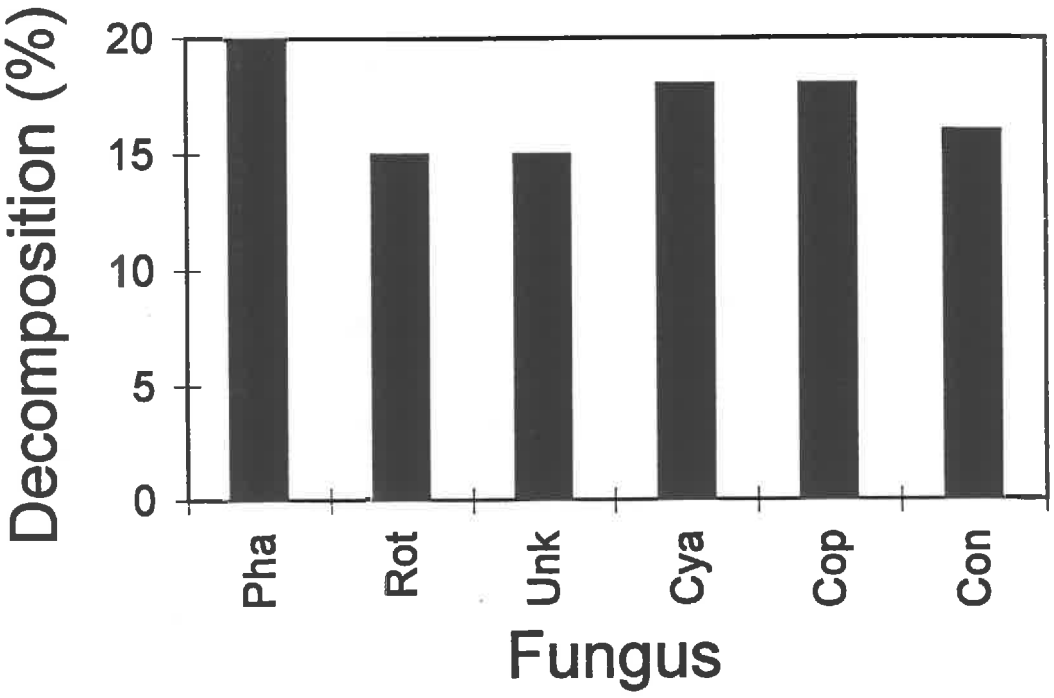

Fig. 6. Percent decomposition of unsterilized straw inoculated with five groups of fungi. There were no significant differences among the groups. All groups inclucding Con caused significant decomposition. Pha = *P. chrysosporium*, Rot = fungi isolated from rotting wood, Unk = unknown fungi isolated from canola straw, Cya = *Cyathus*, Cop = *Coprinus*.

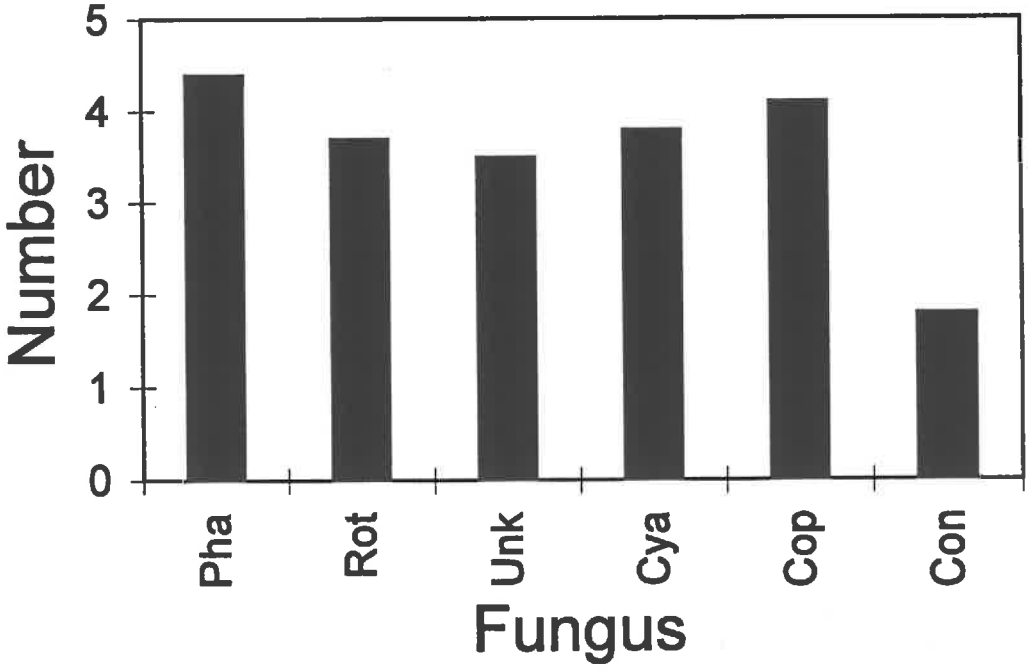

Fig. 7. Number, out of five, of blackleg-infested straws yielding *L. maculans* after inoculation with five groups of test fungi. The treatment effect was significant at 0.056. Pha = *P. chrysosporium*, Rot = fungi isolated from rotting wood, Unk = unknown fungi isolated from canola straw, Cya = *Cyathus*, Cop = *Coprinus*.

- Within the limited scope of environmental conditions tested, urea-sulfuric acid and iron-hydrogen peroxide were ineffective in increasing straw breakdown.
- None of the groups of fungi examined in this study appear likely to result in a practical control for blackleg in the near future. In particular:
    - Although *P. chrysosporium* showed remarkable ability to decompose sterilised canola straw, its intolerance to cool temperatures and its relatively poor ability to decompose unsterilised straw suggest that it has little potential as a biological control agent of blackleg.
    - Somewhat unexpectedly, wood decay fungi, as a group, were not better at decaying sterilized canola straw than fungi isolated from canola straw.
    - The two groups of fungi with the best potential for biological control, among those tested, were the genera *Cyathus* and *Coprinus*. Both were tolerant of cooler temperatures and showed considerable ability to colonize unsterilised straw. However, they did not demonstrate much ability to decompose unsterilised straw or replace *L. maculans* from blackleg infested straw. Nonetheless, a field experiment is now is progress to test their ability to decompose straw in the field.

Literature cited

Christensen, B. T. 1986. Barley straw decomposition under field conditions: effect of placement and initial nitrogen content on weight loss and nitrogen dynamics. Soil Biol. Biochem. 18: 523-529.

Derksen, D. A., Blackshaw, R. E., and Boyetchko, S. M. 1996. Sustainability, conservation tillage and weeds in Canada. Can. J. Plant Sci. 71: 651-659.

Koenigs, J. E. 1974. Hydrogen peroxide and iron: a proposed system for decomposition of wood by brown-rot Basidiomycetes. Wood and Fiber 6: 66-80.

Littell, R. C., Milliken, G. A., Stroup, W. W., and Wolfinger, R. D. 1996. SAS system for mixed models, Cary. NC. SAS Institute Inc. 633 pp.

Smith, J. H., and T. L. Jackson. 1987. Decomposition of wheat and barley straw treated with urea-sulfuric acid. Biology and fertility of soils. 4: 199-204.

Summerell, B. A., and Burgess L. W. 1989. Decomposition and chemical composition of cereal straw. Soil Biol. Biochem. 21: 551-559.