Effect of fungicides on germination of Albugo candida oospores in vitro and on the foliar phase of the white rust disease'

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An oospore germination technique was used to study the effectiveness of 27 fungicides, including some systemic ones, in inhibiting germination at various stages. Among the chemicals tested, Mersil, PMA-10, and Panogen, each at a concentration of 500 ppm active ingredient, inhibited germination about 75%. Of the nonmercurial compounds, mancozeb and ethazole were the best giving about 60% inhibition. Several compounds were tested in the growth chamber for controlling the foliar phase of the disease. Application of either chlorothalonil or mancozeb. at 250 or 500 ppm, respectively, 6 hr before inoculation plus a week after inoculation controlled the disease effectively. However, sprayings of either fungicide 24 hr after inoculation plus a week later were not effective. Two foliar sprayings of chlorothalonil in June significantly reduced both foliar and systemic infections in the field. However, in view of the growth room studies on successful initiation of systemic infections, a third application at the time of flowering is also advised. More studies are required to determine when sprays should be applied for maximum disease control.

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Les oospores hivernant dans le sol ou portees sur les graines constituent l'inoculum primaire des infections de rouille blanche (bois de cerf) sur les cultures de colza de l'Ouest canadien. Nous avons utilise une technique de germination d'oospores pour comparer l'efficacite de 27 fongicides, dont quelques endothérapiques, à divers stades de la germination. Parmi les produits etudies, le Mersil, le PMA-10 et le Panogen, a des concentrations de 500 pprn de principes actifs, ont inhibé la germination dans la proportion d'environ 75 p. 100. Le mancozèbe et l'ethazole ont été les meilleurs des produits non mercuriels, donnant environ 60 p. 100 d'inhibition. Plusieurs composes ont ete evalues en chambre de croissance sur leur efficacité a la phase foliaire de la maladie. Des applications de chlorothalonil a 250 ppm ou de mancozebe a 500 ppm, exécutées 6 h avant et une semaine apres l'inoculation se sont révélées efficaces. Toutefois, ces mêmes produits appliques 24 h apres inoculation et une semaine plus tard ont ete sans effet. Au champ, deux pulverisations foliaires de chlorothalonil en juin ont reduit le taux d'infection foliaire et systemique mais les essais en chambre de croissance sur le declenchement des infections systemiques portent a conseiller un troisième traitement a la floraison. Il faudra poursuivre les recherches pour etablir le calendrier de pulverisation optimal.

Introduction

White rust [*Albugo* candida (Pers. ex Lev.) Ktze.] race 7 (P. H. Williams, pers. commun.) is the most important disease of Polish or turnip rape (***Brassica campestris*** L.) in Western Canada. B. *napus* (Argentine rape) is immune to this race of the disease (17). In 1977, 48% of the total 1.34 million hectares of rapeseed in the three Prairie Provinces was seeded to ***B. campestris*** cultivars, the remainder to B. *napus* (1). In the absence of suitable control measures, losses caused by white rust in recent years have been quite significant. In Saskatchewan, the losses in 1970, 1971, and 1972 were estimated at 3, 6, and 9%. respectively (11). The estimated loss in northern and central Alberta in 1971 was 1.2% (2).

The disease is characterized by white to cream-colored pustules on the underside of leaves. However, the most conspicuous symptom is distortion and hypertrophy of infected inflorescences. These are often called "stagheads"; when mature they consist almost entirely of oospores. Oospores overwintered in soil or carried on seeds (12) most likely constitute the primary inoculum in the spring and early summer. Successful foliar infection is important for the production of secondary inoculum in the form of zoosporangia and perhaps in initiation of systemic infections (14) which lead to the formation of hypertrophied inflorescences. In the absence of cultivars of B. ***campestris*** resistant to white rust, fungicidal control measures could play an important role in reducing losses from this disease. However, to be truly effective a fungicide should control both primary and secondary inoculum.

Chemical control of white rust in rapeseed has received little attention. Perwaiz et al. (10) reported effective control and increased yield in B. ***campestris*** cultivar Sarson following foliar sprays of Polyram (zinc-activated polyethylene thiram disulphide). Among the fungicides reported by various workers to be effective against related species of *Albugo* are zineb (zinc ethylenebisdithiocarbamate), maneb (manganese ethylenebisdithiocarbamate), chlorothalonil (tetrachloroisophthalonitrile),

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Table 1. Percentage inhibition of germination of *Albugo candida* oospores by chemicals at a concentration of 500 ppm active ingredients

Product name*;	Active ingredients % and formulation**:†	Source	Total % inhibition adjusted †
Bayleton	triadimefon[1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazole-1-y1)-2-butanone] 50%, WP	Chemagro	24.1
Benlate	benomyl [methyl-1-(butylcarbamoyl)-2-benzimidazole-carbarnate] 50%, WP	Du Pont	24.5
Bravo	chlorothalonil (tetrachloroisophthalonitrile) 54%, Fwble.	Diamond-shamrock	21.2
Bromosan	thiophanate-methyl [dimethyl-4,4-o-phenylenebis-(3-thioallophanate)] 16.67% + thiram (tetramethylthiuram disulfide) 50%, WP	Cleary	46.2
Calixin	tridemorph (2,6-dimethyl-4-tridecylmorpholine) 75%, Soln	BASF	27.9
Chlorophenate	chlorophenate mixture 18%, WP	Cleary	56.7
Cyprex	dodine (N-dodecylguanidine acetate) 65%, WP	Cyanamid	24.9
Dexon-PCNB	p-dimethylaminobenzenediazo sodium sulfonate 35% + pentachloronitrobenzene 35%, 35%-35%, WP	Chemagro	21.0
Dowco-269	pyroxychlor [2-chloro-6-methoxy-4-(trichloromethyl) pyridine] 97%, Soln	DOW	46.5
DPX 3217	2-Cyano-N-(ethylaminocarbonyl)-2-(methoxyimino) acetamide 50%, WP	Du Pont	55.9
Dwter	fentin hydroxide (triphenyltin hydroxide) 19%, WP	Ciba Geigy	27.9
Kocide-101	copper hydroxide 83%. WP	Kennecott	13.2
Manzate-200	mancozeb (zinc and manganese ethylene-bisdithio-carbamate) 80%. WP	Du Pont	59.8
Mersil	mercury chloride ($HgCl_2$) 14% + mercurous chloride (Hg_2Cl_2) 28% + mercury equivalent 34%, WP	May and Baker	75.7
N.F. 48	thiophamine [2-(3-methoxycarbonyl-thioureido)-analine] 80%, WP	Nippon Soda	22.5
N.F. 65	thiophamine 40% + bis-(dimethylthiocarbomoyl disulfide) 40%, 80%, WP	Nippon Soda	25.5
Panogen	methylmercury dicyandiamide 0.9%, WP	Morton	74.5
PMA-10	phenyl mercuric acetate 10%, Soln	Later	75.7
Polyram	metiram [ammoniates of ethylene-bis-dithiocarbamate zinc 83.9% + ethylenebis-dithiocarbamic acid)] 16.9%, WP	Niagara	37.5
Sicarol	pyracarbolid (2-methyl-5,6-dihydro-4H-pyran-3-carboxanilide) 50%, WP	Hoechst	19.8
Tersan SP	chloroneb (1,4-dichloro-2,5-dimethoxybenzene) 65%, WP	Du Pont	22.0
Terraclor	quintozene (pentachloronitrobenzene) 75%, WP	Olin	20.4
Terrazole	ethazole [5-ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole] 35%, P	Olin	59.8
Topsin M	thiophanate methyl [dimethyl 4,4-o-phenylenebis-(3-thioallophanate)] 70%. WP	Pennwalt	14.8
Vitavax	carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) 75%, WP	UniRoyal	28.8
R-28921	O,O-diethyl-2-[(3-methoxycarbonyl)thioureido] phenyl phosphoramidothioate 50%, WP	Stauffer	21.2
LFA 2043	iprodione	May and Baker	21.5
Control †			

* The use of trade names in this publication does not imply endorsement by Agriculture Canada of the products named or criticism of similar ones not mentioned.

** WP = wettable powder, P = powder, Soln = solution, Fwble - flowable.

† At least 400 spores counted per sample. Percentages of nongerminated oospores in the control (18) were subtracted from those in the treatments to obtain percentage inhibition due to fungicide.

dodine (N-dodecylguanidine acetate), and captan (N-[(trichloromethyl)thiol]-4-cyclohexene-1,2-dithiocarboximide) (3, 4, 7, 10). This paper presents results on the *in vitro* sensitivity of *A. candida* oospores to various fungicides and on the control of the foliar phase of white rust disease of *B. campestris* in the growth chamber and field.

Materials and methods

Screening for inhibition of oospore germination

Twenty-seven fungicides were tested for inhibition of oospore germination (Table 1). Concentrations of 500 ppm active ingredient (a.i.) were used for all fungicides. To obtain oospores for fungicide trials, dry hypertrophied

tissue collected from B. ***campestris*** plants infected with race 7 of A. ***candida*** in 1972 was finely ground with a mortar and pestle and screened through a 60-mesh sieve.

The method employed for testing the effects of chemicals on oospore germination was similar to that reported previously (16). Fifty ml sterile tap water solution or suspension of each test chemical was placed in a 125-ml erlenmeyer flask and a small amount of the oospore powder added. The mixtures of chemical, sterile tap water and oospores, or only sterile tap water and oospores for the control, were incubated at 18-20°C on a rotary shaker (200 rpm) for one week. The spore suspension was then poured into a Petri dish and kept stationary for a period of 24 hr at 13°C. Samples from each Petri dish were placed on slides in lactophenol aniline-blue. Counts of germinated oospores were made at magnification of 800X under oil. Percentages of non-germinated oospores in the control were subtracted from those in the treatments to determine the percentage inhibition due to the fungicide. Fig. 1 (A-D).

Growth chamber experiments

Some chemicals were also tested for controlling the foliar phase of the disease. Plants of ***B. campestris*** cultivar Torch were grown in modified Cornell soilless mix (15) and were maintained under 18 hr illumination (17,000 lux) at 21°C with a night temperature of 16°C. Two weeks after seeding, cotyledons and leaves of all experimental plants were drop-inoculated with a zoospore suspension obtained from germinating zoosporangia of A. ***candida*** race 7. Hemacytometer counts of zoospores in the suspensions ranged from 100,000 to 150,000 per ml. Control plants were drop-inoculated with sterile tap water. Following a dark period of 24 hr,

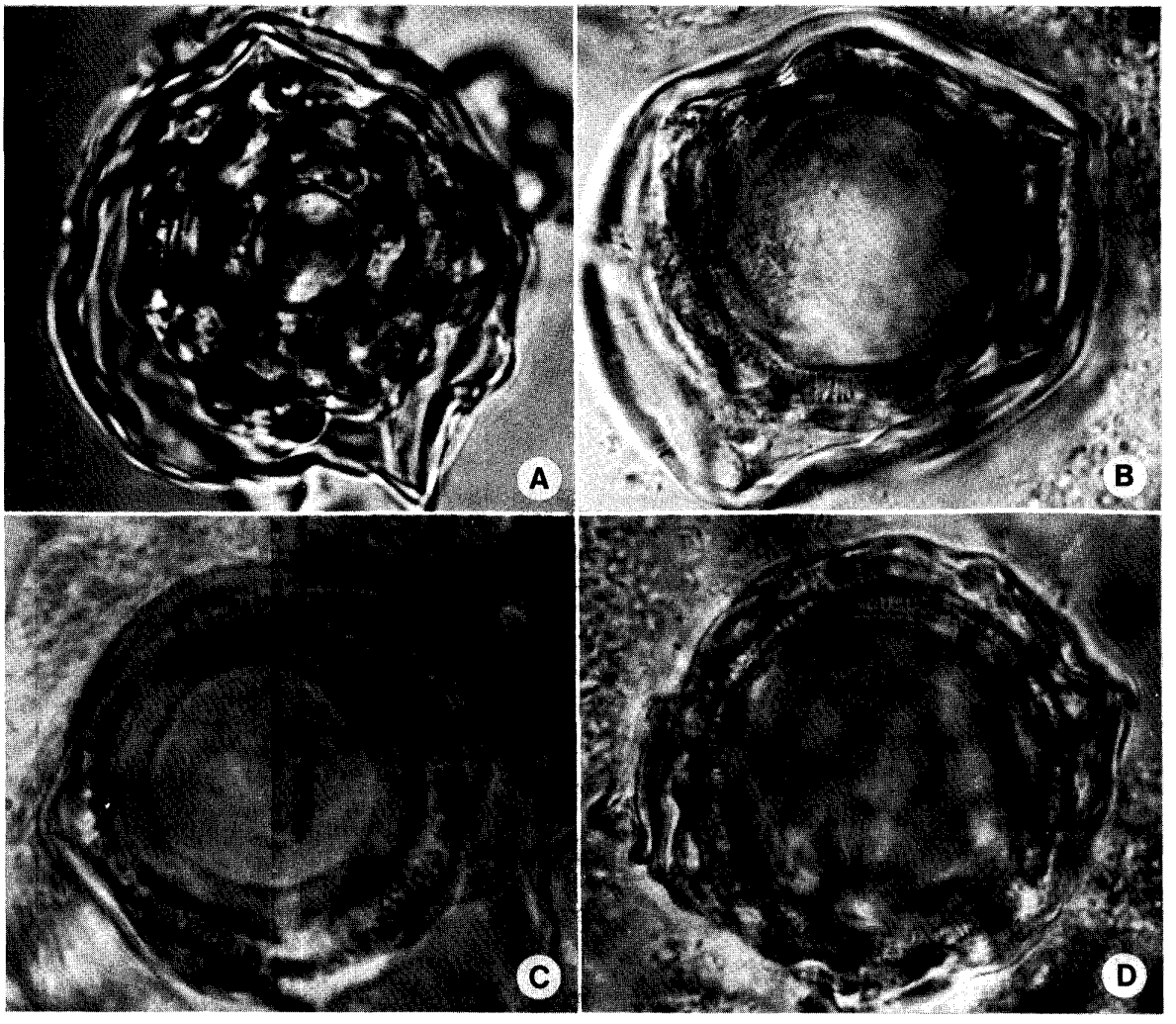

Figure 1 (A to D). Nongerminated oospores (A to C): (A) with both granular contents and central body, (B) with granular contents only and (C) both granular contents and central body almost disappeared. Germinated oospore, (D) both granular contents and central body absent.

plants were returned to an 18-hr day and maintained under a continuous water mist for a period of 3-4 days.

Preliminary screening of nine fungicides in the growth chamber showed that chlorothalonil and mancozeb were the only chemicals showing promise and therefore, subsequent experiments were conducted only with these two. The two concentrations used for chlorothalonil and mancozeb, respectively, were 100 and 250 and 250 and 500 ppm active ingredient. The plants were sprayed on both sides of leaves to run-off. Tween-20 was used at the rate of 1 ml per 100 ml of the fungicide solution.

There were five schedules for each concentration of fungicide as follows: Schedule 1: The first application was 96 hr before inoculation when the plants were 10 days old. Additional applications were made 24 hr before, and 96 and 168 hr after inoculation. Schedule 2: Applications were made 24 hr before, and 96 and 168 hr after inoculation. Schedule 3: Fungicides were sprayed 6 hr before and 168 hr after inoculation. Schedule 4: Applications were made 24 hr and 168 hr after inoculation: Schedule 5: Control sprayed with water. Two or three plants were grown in each 10-cm pot and there were five pots of each schedule of each fungicide.

Two weeks after inoculation the numbers of pustules on all infected leaves were counted. The experiment was replicated at three different times and the results presented are the mean of all three replicates.

Because the dependent variables are dichotomous (i.e. qualitative or categorical data) the data were analysed by the maximum likelihood procedure described by Fienberg (5) and Goodman (8) and used more recently by Gavora et al. (6). Since the data were in the form of a table of counts, a four-dimensional contingency table with the categories being replicate, fungicide treatments, time of application and infection, maximum likelihood estimates of the expected frequencies were carried out for a number of models. The appropriate $\log_e$ likelihood ratio statistic was calculated for each of these models. This statistic approximately follows the chi-square distribution which was used to test hypotheses on how well the various models described the data.

Field experiments

Results of growth chamber tests and a preliminary unreplicated field test in 1975 showed that chlorothalonil might be an effective protectant for the control of A. *candida* on rapeseed. Therefore, in 1976, a field study with four different spray schedules and a nonsprayed check was established to determine how chlorothalonil could be effectively utilized. Each plot consisted of eight 6.6-m rows spaced 32 cm apart. All treatments were replicated four times in a randomized block design with 2-m pathways between the ends of the plots. Certified seed of B. *campestris* cv. Torch was sown on May 19 at the rate of about 350 seeds per row. Carbofuran 5G was applied with the seed at the rate of 1 g per row for flea beetle (*Phyllotetra* spp.) control. Chlorothalonil (54% a.i.) was mixed with water and applied with a low-pressure hand sprayer. Spray volume was 1.3 kg a.i./550 litres/hectare. The four spray schedules were: No. 1 (June 9 and 17), No. 2 (June 9, 17, and July 9), No. 3 (June 9, 17, July 9, and 23), No. 4 (June 9, 17, 24, July 9, 16, and 23). Applications were always made on calm mornings or evenings.

Both on June 30 (growth stage 3.1) and July 15 (growth stage 4.2) fifty plants randomly pulled from each plot were rated for number of plants and leaves infected and number of pustules per infected leaf. When plants were approaching maturity on August 16 (growth stage 5.4), 100 plants were randomly pulled from each plot and rated as to presence and number of stagheads. All the remaining plants in a plot were harvested on September 8 to obtain yield data.

Results

Of the 27 chemicals tested, the three mercurial fungicides, Mersil, PMA-10, and Panogen, were the best inhibitors of oospore germination (Table 1). The total percentage inhibition with any of these fungicides was about 75. Among the nonmercurial compounds, mancozeb and ethazole were the most effective giving total inhibition of about 60%. The inhibition provided by Bromosan or Pyroxychlor was about 50%. All other compounds listed in Table 1 were not very effective at the concentration tested.

Preliminary screening of nine fungicides in the growth chamber showed that carbathiin, chlorophenate and benomyl were phytotoxic causing stunting and leaf-tip burning. Polyoxin AGB, Polyoxin B, Pyroxychlor and DPX 3217 were relatively ineffective in controlling the foliar phase of white rust. Chlorothalonil and mancozeb were the only chemicals showing promise and therefore, only results of these two are presented here.

Since all of the plants from the control and nearly all from spray schedule 4 were infected, the multidimensional contingency table analysis was done using only data from the first three spray schedules. Log_e likelihood ratio statistics are given in Table 2. The results in Tables 3 and 4 suggest that irrespective of time of application the differences in the degree of control between schedules 1, 2, and 3 were not significant; differences between schedule 4 and the control were not significant, but they were both significantly different from other spray schedules. However, the percent control obtained with two rates of application of both fungicides in schedules 1, 2, and 3 was significantly different (Table 2).

In schedules 1, 2, and 3 about 50% of the plants sprayed with chlorothalonil at 100 ppm showed white rust symptoms 14 days after inoculation, as compared to very little disease on those sprayed at 250 ppm (Table 3). However, regardless of concentration, the amount of

Table 2. Multidimensional contingency table analysis of numbers of clean and white rust-infected plants pooled over three schedules of fungicide sprays on turnip rape in the growth chamber

Source of variation	df	-2XLLR*
Replicate x fungicide x schedule	12	5.905
Fungicide x schedule	6	0.835
Replicate x schedule	4	0.756
Replicate x fungicide	6	1.915
Schedule	2	2.735
Fungicide	3	82.223**

* -2XLLR = the negative of two times the log likelihood ratio

** Statistically significant difference, P = 0.01

disease on plants sprayed 24 and 168 hr after inoculation was about the same as that in the control. The mean number of pustules per infected leaf on plants sprayed as per schedule 4 and in the control were 12 and 13, respectively (Table 3).

Mancozeb was not as effective as chlorothalonil in preventing white rust infection (Table 4). Even plants sprayed at 500 ppm in schedules 1, 2, and 3 developed some white rust symptoms. However, as for chlorothalonil, plants sprayed 24 hr after inoculation and then a week later, developed as much disease as that recorded in the control (Table 4).

In the field, chlorothalonil effectively reduced foliar and systemic infections when applied as a foliar spray (Table 5). Two sprays in June (June 9 and 17) significantly reduced disease severity and increased yield slightly. Results of June 30 sampling are not included in Table 5 because of the fact that only one out of 50 plants in the check had developed white rust symptoms; all sampled plants from spray treatments were free of disease. Data recorded on July 15 and August 16 show that percentages of infected plants, number of infected leaves per plant, number of pustules per leaf, and percentages of plants with stagheads were invariably higher in the check than those on plants from sprayed treatments (Table 5). The differences between the four spray treatments for the four variables were not significant in most cases. However, with the exception of mean number of infected leaves per plant, the unsprayed check was significantly different from other treatments in all cases. The total grain yield in the four spray treatments was about 10% higher than in the check. However, because of variability within treatments, the differences were not significant.

Discussion

Albugo candida oospores occur commonly on ***Brassica*** seed samples throughout the Prairies (12). According to this report the inoculum levels on seeds may be considerably higher than actually required for initiation of infection bearing in mind that on germination a single oospore releases 40-60 zoospores (13, 16). Recent reports have demonstrated oospore germination following a period of washing in water (13, 16) and infection of ***Brassica*** cotyledons by zoospores produced from germinating oospores (17). Furthermore, unpublished data from field experiments showed more foliar and systemic infections in plots where the seed was treated with oospore powder than in the controls. This evidence supports the view that seed-borne oospores constitute

Table 3. Efficacy* of Bravo applied at two concentrations in four spray schedules against A. ***candida*** on turnip rape in the growth chamber

Spray schedules			Concentration applied a.i.	Mean % plants infected	Mean no. of pustules/leaf
Number	Hours from inoculation Before	After			
1	96 + 24	96 + 168	100	44.1	7.3
			250	6.7	3.0
2	24	96 + 168	100	52.9	7.1
			250	11.1	2.6
3	6	168	100	50.0	7.7
			250	5.6	2.0
4	-	24 + 168	100	100.0	12.0
			250	97.4	12.2
Control	-	-	-	100.0	13.0

* Recorded 14 days after inoculation

Table 4. Efficacy* of Manzate-200 applied at two concentrations in four spray schedules against A. ***candida*** on turnip rape in the growth chamber

Spray schedules					
	Hours from inoculation		Concentration applied	Mean % plants	Mean no. of
Number	Before	After	a.i.	infected	pustules/leaf
1	96 + 24	96+ 168	250	47.6	5.7
			500	10.5	1.9
2	24	96+ 168	250	56.8	6.3
			500	18.2	2.3
3	6	168	250	45.7	5.0
			500	11.8	5.6
4	–	24+ 168	250	100.0	10.7
			500	100.0	11.5
Control	–	–	–	100.0	13.0

* Recorded 14 days after inoculation.

Table 5. Efficacy of Bravo in four spray schedules against A. ***candida*** on turnip rape in the field, 1976

			July 15			Aug. 16	Sept. 8
Schedules	No. of sprays	Dates applied	% infected plants	Mean no. of infected leaves/plant	Av. no. of pustules/leaf	% plants with stagheads	Mean yield/plot (g)
Check	–	–	76.0 a*	1.76 a	7.9 a	14.3 a	2485 a
1	2	June 9, 17	19.0 b	1.37 a	3.1 b	2.5 b	2776 a
2	3	June 9, 17 and July 9	21.5 b	1.33 a	3.1 b	2.3 b	2763 a
3	4	June 9, 17 and July 9, 23	17.5 b	1.51 a	3.6 b	1.3 b	2765 a
4	6	June 9, 17, 24 and July 9, 16, 23	3.5 c	1.43 a	2.0 b	1.8 b	2674 a

* Within column, figures suffixed by the same letters do not differ significantly at the 5% level. as determined by Duncan's multiple range test.

primary inoculum for infection of ***Brassica*** species in western Canada. Thus seed treatment even by a protectant fungicide could be important in controlling white rust infections either by inhibiting oospore germination or by killing the zoospores on emergence. None of the fungicides tested in the present study was 100% effective. The mercury fungicides were found to be the best inhibitors of oospore germination, but provided only 75% inhibition. Therefore, the search for a completely effective fungicide, preferably a systemic, needs to be continued.

The growth chamber experiment showed that chlorothalonil or mancozeb at 250 and 500 ppm active ingredients, respectively, applied 6 hr before inoculation and then a week later, controlled the disease effectively without any apparent phytotoxic effects. In view of their mainly protectant action, failure to control white rust by either fungicide applied 24 hr and 168 hr after inoculation was not surprising, because establishment of A. ***candida*** infection of rapeseed cotyledons and perhaps on leaves would normally be completed within 24 hr of inoculation (17).

Two years' field experiments showed that chlorothalonil possesses sufficient protectant activity to control white rust in B. campestris cultivar Torch. These results are in agreement with those of Chambers et al. (3) where chlorothalonil was also found effective for control of white rust of spinach. Two sprays in June when the plants were about 3-4 weeks old reduced the disease significantly.

Rain fell on 17 of the 23 days between June 22 and July 14. The high humidity during this period probably resulted in a build-up and spread of inoculum. The data suggest that application of chlorothalonil on June 24 at the beginning of this period was perhaps at a time prior to release and spread of inoculum and was therefore effective in reducing infection. Should that be the case, it lends support to our growth chamber experiments where an application 24 hr before inoculation was quite effective. The data also suggest that an additional spray treatment of chlorothalonil in late July could result in a slightly lower incidence of floral infection.

The distortion and hypertrophy of infected inflorescences (staghead) is the most important factor from a yield-loss standpoint (9). Cotyledonary infections in the spring have been considered responsible for the formation of stagheads through systemic infections (14). However, growth chamber experiments have shown that large numbers of staghead can be initiated at a later stage of plant development by infecting floral buds (unpublished data). In view of these findings, it is possible that significant reduction in the number of stagheads on plants in sprayed treatments over the control was due to control of foliar and floral infections.

Failure to demonstrate significant yield responses to disease control in field tests was not surprising. However, if evaluated on a larger scale with high plant densities and disease intensity, such as is commonly observed in commercial fields, economic yield responses would probably result from effective disease control. More studies are required to determine when fungicides should be applied to ensure maximum disease control. The results do indicate that two applications in June and an application at the time of flowering are required to reduce the disease to a significant level. However, multiple application may not be economically feasible under commercial rapeseed production.

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