IMPROVING THE PROTECTIVE FUNGICIDAL ACTIVITY OF NICKEL SULFATE AGAINST LEAF RUST OF WHEAT AND CROWN RUST OF OATS[1]

F. R. Forsyth and F. Jursic

Abstract

A marked improvement in the retention of protective activity of 400 p.p.m. nickel sulfate hexahydsate against leaf rust of wheat and crown rust of oats was noted when the non-ionic surfactant Triton X-114 at 0.1% or the anionic surfactants sulfonated castor oil at 0.1% or a constituent of Atlox G-3300 at 0.25% were included in the solution. The polybutene Indopol L-10 at 0.25% and the petroleum oil Imperial Oil 862-B at 0.3% (the latter for leaf rust only) were also effective, This activity persisted after the application of one-half inch of simulated rain. Less improvement was shown with the; addition'of the non-ionic surfactants Triton X-100 at 0.1% and Triton B-1956 at 0.08%, the anionic surfactant dodecylbenzene sodium sulfonate at 0.25% or the cationic surfactant Hyamine 3500 at 0.03%. The chemicals Indopol L-10 at 0.75%, dodecylbenzene sodium sulfonate at 0.25%, the constituent of Atlox G-3300 at 0.25%, Hyamine 3500 at 0.03% and the petroleum oil Imperial Oil 862-B at 0.3% were fungicidal in the absence of nickel sulfate. The protective activity of the Indopol L-10, the Imperial Oil 862-B and dodecylbenzene sodium sulfonate, all used with nickel, persisted through the application of one-half inch of rain.

Introduction

Inorganic salts of nickel have been used (5) to control wheat leaf rust (Puccinia recondita Rob. ex Desm.) on Thatcher, Marquis and Red Bobs wheat in the field. The success of the nickel salts in field use seemed attributable to their ability to eradicate the rust infections present in the leaf and not to any protective action of the nickel. This was somewhat surprising since nickel compounds had been shown to be effective as protective fungicides against leaf rust in the greenhouse (5) at concentrations one-fourth of those required for eradicative action. However, it had been shown (5), (6) that the protective action of the nickel was rather easily removed with simulated rain. Various surfactants had been used in studies (5), (6) and (7) of nickel as a fungicide but no systematic study had been made of the relative effectiveness of spray additives in improving the protective fungicidal activity of the nickel ion. The present study is a report of greenhouse experiments designed to compare the effectiveness of various compounds, mainly surface ,active agents, in enhancing the protective action of inorganic nickel compounds both with and without simulated **rain.**

[1] Contribution No. 191 from the Pesticide Research Institute, Canada Department of Agriculture, Research Branch, London, Canada,

The polybutene (Indopol L-10) was included because of the report (4) that the polybutenes control powdery mildew of roses (Sphaerotheca pannosa (Wallr. ex Fries) Lkv.

The Imperial Oil 862B was included since similar oils are known to be efficient (2) in controlling Mycospharella musicola Leach (Cercospora musae Zimm.) and because it had shown some promise as a protective fungicide against the cereal rusts in preliminary field tests at Winnipeg.

Methods

Plant material was obtained by growing Thatcher wheat or Victory oat seedlings in soil in 4-in, earthenware pots. When the first leaves of the seedlings were about 3 in. long they were used in the test of protective activity. The fungicides were applied to the surface of the seedling leaves by atomizing 100 ml of solution onto the seedlings in 6 of the pots. There were 8 - 10 seedlings in each pot. A paint sprayer nozzle operated by compressed air was used to direct the atomized spray onto the seedlings as the pots were rotated on a turn-table. The seedlings were allowed to dry before rust urediospores were sprayed on with a second spray nozzle. The urediospores were dispersed in a water solution by shaking rusted leaves in a weak solution of sulfonated castor oil in water (3 drops per 100 ml). The urediospores in water were applied at the rate of 50 ml to 20 pots of seedlings. The seedlings were then incubated for 24 hours in a moist chamber. The number of uredia on each leaf was counted 10 days later.

The average number of infections per untreated leaf varied from one experiment to the next, the range being between 50 and 200. To present the data as percentage infection the values have all been adjusted to correspond with a value of 100 infections per leaf on the untreated plants. For example, if the average number of infections per leaf on the untreated leaves in an experiment was 50.0 and for treatment A it was 3.0 then each value would be multiplied by 2 and the values entered in the table as 100 for the untreated and 6.0 for treatment A.

In experiments to determine the effective life of the fungicide on the leaf surface it was necessary to mark each leaf at the time of application by searing the leaf with the tip of a small soldering iron at a point approximately 1 cm above the base of the leaf blade. Whenever counts were made of the number of infections per leaf only the infections above this necrotic .spot were counted.

Distilled water directed with a pressure of 10 pounds/sq. in., through a nozzle giving a fan shaped spray pattern, was used to simulate rain. The spray was directed on the seedlings as the pots rotated on the turn-table. A Petri dish marked at the one-half inch depth rotated with the seedlings and was at the same height as the soil surface in the pots. When the spray water was one-half inch deep in the dish it was considered that the seedlings had received the equivalent of one-half inch of rain.

Atlox G3300 (supplied by Atlas Powder Company, Canada Ltd., Brantford, Ont.), mentioned in the Tables, was actually a fraction of this compound. The Atlox, in water solution, was extracted with petroleum ether and the water soluble fraction was passed through the Dowex 50 W X 8 ion exchange resin column. The material that passed through the Dowex 50 was evaporated to dryness and used as the additive. The fraction was presumably a sulfonic acid constituent of the original mixture.

Santomerse I (obtained from Monsanto Canada Ltd., Montreal, Que.) was extracted with absolute ethyl alcohol and the alcohol was filtered to remove sodium sulfate. The ethyl alcohol was removed under vacuum and the material remaining, presumably dodecylbenzene sodium sulfonate, was used as the spray additive.

A 25% stock solution of Indopol E-10 (supplied by R. J. Brown of Canada Ltd., Toronto, Ont.) was prepared by mixing 50 ml xylene, 50 ml Indopol L-10, 1 ml Tween 81 and 99 ml water.

Triton X-114 (a water soluble octylphenoxypolyethoxy ethanol) and Triton X-100 (a water soluble iso-octylphenoxy polethoxy ethane) were considered to be 100% active and were used as supplied by the Rohm and Haas Co. of Canada, West Hill, Ont. Triton B-1956 (modified phthalic glycerol alkyd resin, from the same source was considered to be 77% active. Hyamine 3500, also from Rohm and Haas Co. of Canada, is a 50% aqueous solution of a selected blend of alkyl dimethyl benzyl ammonium chlordies.

The Imperial Oil fraction 862B (a petroleum oil fraction) was obtained from Imperial Oil Ltd., Sarnia, Ont. A 25% stock solution was prepared as for Indopol L-10,

Results

Surfactants and Indopol L-10 as additives: The first spray additive in these tests to prove effective' in enhancing the 'protective action of solutions of inorganic salts of nickel was sulfonated castor oil. Various other additives were then tested along with the nickel sulfate against race 5a of leaf rust of wheat and the results are presented in Table 1. The numbers given are the averages from three separate experiments. The surfactants and Indopol L-10 with and without nickel were all excellent protective fungicides in the absence of rain. This has little practical significance except in the control of foliage diseases in the greenhouse. On the other hand the persisting protective action of many of these solutions after exposure to one-half inch of simulated rain was significant. This demonstrated the feasibility of using nickel salts as protective fungicides providing the proper surfactant or other additive was used.

The Indopol k-10 at 0.75% was a good protective fungicide against leaf rust of wheat when used alone. However, it also was an aid in maintaining the activity of the nickel since whenever the L-10 at 0.25% was used alone it did not give reliable protection but when used with 400 p.p.m. nickel sulfate an,effective protective fungicide was produced,

The Santomerse at 0.25% proved to be an effective fungicide against leaf rust with and without rain. When it was combined with nickel the protective action in presence of rain was partially lost.

The fact that there are only about 19 infections per leaf on the nickel sulfate treated leaves after rain washing may seem to indicate considerable efficiency on the part of the inorganic salt alone. However, the presence of 19 infections on a wheat leaf provides such a large amount of inoculum that subsequent increase in the number of infections is very rapid. Probably an average of not more than 1.0 infection per leaf should be considered successful protection,

Table 1. The protective activity of nickel sulfate combined with surfactants, Indopol L-10 or Imperial Oil 862-B against leaf rust on Thatcher wheat.

Chemical			Percent Infection		Phytotoxicity
			No rain	1/2" rain	
Nickel sulfate[a] 400 p.p.m.	+ Triton X-114	0.1%	0.9[b]	0.2	slight
"	+ Triton X-100	0.1%	0.3	2.2	"
"	+ Triton B-1956	0.08%	0.1	6.0	negligible
"	+ sulfonated castor oil	0.1%	0.0	0.4	"
"	+ castor oil	0.1%	0.8	18.4	"
"	+ Atlox G-3300	0.25%	0.0	0.6	"
"	+ Santomerse I	0.25%	0.0	3.9	slight
"	+ Hyamine 3500	0.03%	0.0	2.3	negligible
"	+ Indopol L-10	0.25%	0.0	1.1	"
"	+ Indopol L-10	0.75%	0.0	0.7	"
"	+ Imperial Oil 862-B	0.3%	0.0	0.4	"
Indopol L-10 0.25%			15.3	9.4	"
Indopol L-10 0.75%			1.0	0.6	"
Santomerse I[c] 0.25%			0.0	0.3	slight
Atlox G-330[d] 0.25%			0.3	15.4	negligible
Hyamine 3500 0.03%			1.7	11.8	"
sulfonated castor oil 0.1%			76.3	68.3	"
Imperial Oil 862-B 0.3%			0.7	0.9	"
Triton X-100 0.1%			100.0	100.0	slight
Nickel sulfate 400 p.p.m.			1.88	19.3	negligible
Untreated			100.0	100.0	

[a] Nickel sulfate as $NiSO_4 \cdot 6H_2O$

[b] Each value is the average number of infections per leaf, based on counts of 90 leaves in three separate experiments. All averages have been adjusted on the basis of the untreated leaf having 100 infections.

[c] The dodecylbenaene sodium sulfonate obtained from Santomerse I was used.

[d] The constituent of G-3300 obtained as described in the methods was used,

Table 2. The protective activity of nickel sulfate combined with surfactants or Indopol L-10 against crown rust on Victory oats.

Chemical			Percent Infection		Phytotoxicity
			No rain	1/2" rain	
Nickel sulfate[a] 400 p.p.m.	+ Triton X-114	0.1%	0.7	1.4	medium toxicity
"	+ Triton X-100	0.1%	0.1	0.8	very slight toxicity
"	+ Triton B-1956	0.08%	0.6	1.6	negligible
"	+ sulfonated castor oil	0.1%	1.5	2.0	"
"	+ Atlox G-3300	0.25%	0.0	0.7	reduced growth
"	+ Santomerse I	0.25%	0.0	-	negligible
"	+ Hyamine 3500	0.03%	0.0	-	"
"	+ Indopol L-10	0.25%	0.5	1.4	"
Indopol L-10 0.25%			12.3	4.5	"
Indopol L-10 0.75%			0.3	0.3	"
Santomerse I[b] 0.25%			0.4	2.1	"
Atlox G-3300[c] 0.25%			0.0	22.0	"
Hyamine 3500 0.03%			0.6	-	"
Nickel sulfate 400 p.p.m.			6.3	26.1	"
Untreated			100.0	100.0	"

[a] Nickel sulfate as $NiSO_4.6H_2O$

[b] The dodecylbenzene sodium sulfonate obtained from Santomerse I was used.

[c] The constituent of G-3300 was used.

The data presented in Table 2 shows that the activity of the surfactants and Indopol L-10 with and without nickel was about the same when used in solutions against crown rust of oats (*Puccinia coronata* Corda f. sp. *avenae* Erikss.) as against leaf rust of wheat.

Sulfonated castor oil as an additive for other metal salts:

Since the sulfonated castor oil was so effective in enhancing the activity of nickel, experiments were carried out to compare the activity of nickel, copper, zinc and manganese sulfate salts along with sulfonated castor oil against leaf rust of wheat and crown rust of oats. The order of effectiveness was the same for both organisms, with and without rain; nickel > copper > zinc > manganese. Of the metal salts tested in the presence of sulfonated castor oil only the nickel would be considered an efficient protective fungicide with and without simulated rain.

Table 3. Protective activity of copper, zinc, manganese and nickel sulfate salts with sulfonated castor oil

Chemical		Percent Infection: Leaf rust of wheat, No rain	Leaf rust of wheat, 1/2" rain	Crown rust of oats, No rain	Crown rust of oats, 1/2" rain	Phytotoxicity
Copper sulfate[a] 200 p.p.m,						
t sulfonated castor oil	0.1%	3.2	18.1	7.0	7.5	negligible
Zinc sulfate[b] 400 p.p.m.						
+ sulfonated castor oil	0.1%	9.0	41.9	9.7	27.5	"
Manganese sulfate[c] 400 p.p.m.						
t sulfonated castor oil	0.1%	22.3	48.8	21.6	41.2	"
Nickel sulfate[d] 400 p.p.m,						
t sulfonated castor oil	0.1%	0.0	0.6	0.0	0.5	"
Nickel sulfate 400 p.p.m.		25.8	55.5	21.7	34.4	"
Untreated		100.0	100.0	100.0	100.0	

[a]Copper sulfate as $CuSO_4.5H_2O$, [b]Zinc sulfate as $ZnSO_4.7H_2O$
[c]Manganese sulfate as $MnSO_4.H_2O$, [d]Nickel sulfate as $NiSO_4.6H_2O$

Table 4. Protective activitv of nickel sulfate and Indopol L-10 against leaf rust of wheat over a five day period

Chemical		Percent Infection — Day of inoculation with rust, 0 being the day of application of the chemical: 0	0 + 1	o t 2	o t 3	0 + 4	0 + 5
Nickel sulfate 400 p.p.m.		0.0	8.1	11.7	13.8	17.7	9.1
Nickel sulfate 400 p.p.m.							
+ sulfonated castor oil	0.1%	0.0	4.9	0.5	9.4	1.5	0.5
Nickel sulfate 400 p.p.m.							
t L-10	0.75%	0.0	0.7	0.3	0.4	0.3	1.0
L-10	0.75%	0.3	4.5	0.3	0.3	1.3	0.9
Untreated		100.0	100.0	100.0	100.0	100.0	100.0

Protective activity during a 5 day period:

The results reported in Tables 1-4 show the effectiveness of sulfonated castor oil and L-10 as additives for increasing the retentive properties of nickel. The method used demonstrated protective activity for only a few hours because the chemical was applied, the leaves were dried, rainwashed and then inoculated all on the same day. This was not a reliable indication of the stability of the protective action over a period of several days.

In Table 4 the results are presented to show the protective action persisting up to five days after the chemical was first applied to the leaf surface. The nickel sulfate plus sulfonated castor oil, nickel plus L-10 at 0.75% and L-10 alone at 0.75% all retain the protective action against leaf rust of wheat for a period of five days. The L-10 at 0.75% by itself appears to be an efficient protective fungicide, In fact the activity of nickel plus L-10 could be accounted for by the activity of the L-10 alone. However, it will be remembered (Table 1) that the L-10 does aid in the retention of protective activity of nickel.

The next question to answer was whether or not, after a period of weathering in the greenhouse followed by a one-half inch of simulated rain, there would still be sufficient nickel or L-10 on the leaf to protect against leaf rust. In Table 5 the results are shown for the same chemicals as in Table 4 except that in each instance one-half inch of simulated rain was applied three hours before the rust was applied whether this was 0, 1, 2, 3, 4 or 5 days after the chemical was applied.

Table 5. Protective activity of nickel sulfate and Indopol L-10 over a five day period against leaf rust of wheat, 1/2" of rain applied.

Chemical		Percent Infection Day of inoculation with rust[a], 0 being the day of application of the chemical					
		0	0 + 1	0 + 2	0 + 3	0 + 4	0 + 5
Nickel sulfate 400 p.p.m.		33.9	22.5	35.4	48.5	37.0	24.9
Nickel sulfate 400 p.p.m. + sulfonated castor oil	0.1%	1.6	0.6	1.3	3.4	1.4	4.4
Nickel sulfate 400 p.p.m. + L-10	0.75%	0.1	0.05	0.9	0.4	0.14	0.2
L-10	0.75%	0.2	0.7	1.3	0.9	2.3	0.a
Untreated		100.0	100.0	100.0	100.0	100.0	100.0

[a]The plants were washed with 1/2" of rain, allowed to dry and then were inoculated.

The nickel sulfate with sulfonated castor oil or with L-10 and the L-10 alone at 0.75% provided effective protection even after one-half inch of rain. This rain could be applied from 0-5 days after the application of the chemicals without removing the protective action. There are a few instances in Table 5 of more than the theoretical maximum of 1.0 infection per leaf, for effective protective action, but the improvement in tenacity of the nickel with the additives is obvious.

Discussion

The possibility existed that one or more of the surfactants or additives used in this study would interfere with the protective activity of nickel sulfate even in the absence of rain. During the incubation period of inoculated seedlings there is a certain amount of drip-off of water from the leaves. This might have been accentuated by the presence of surfactants. However, the results indicate no such interference. On the contrary a decided improvement in the retention of protective activity of nickel sulfate in the presence of rain was found with added Triton X-114, sulfonated castor oil, Atlox G3300 and Indopol L-10. Less improvement was shown with added Triton X-100, Triton B-1956, Santomerse I and Hyamine 3500. In the case of the surfactants the most logical explanation for this added protection is the formation of a complex between the nickel and the additive. The Indopol L-10 and Imperial Oil 862B may act by a physical interference with the washing action of the rain on nickel sulfate. Furthermore, at the concentrations used, Indopol L-10, Santomerse I, Atlox G3300, Hyamine 3500 and Imperial Oil 862B were fungicidal when used without nickel sulfate.

The fact that Indopol L-10 at 0.75%, Santomerse at 0.25% and Imperial Oil 862B at 0.3%, all showed effective protective action in absence of nickel and after simulated rain is of considerable practical interest. Fisher (4) reported that a 5% emulsion of L-10 provided excellent protection from infection with powdery mildew of cucumbers in thegreenhouse. The 0.75% emulsion of L-10 required here is less than that required to protect against mildew but is nevertheless a high concentration of fungicide. The low cost of the polybutenes allows further consideration of these compounds as possible fungicides. The L-10 and L-100 polybutenes were found in preliminary tests to be superior to the more viscous polybutenes in controlling leaf rust of wheat and crown rust of oats. More detailed information about the comparative effectiveness of the polybutenes will be published at a later date,

Santomerse as used here was the sodium salt of dodecylbenzenesulfonic acid, It has consistently provided somewhat better protection against the rusts when used alone than when used with nickel compounds in the presence of simulated rain. It will be interesting to compare the mode of action as a fungicide (of this anionic surfactant) with that of the cationic surfactants (e.g. dodine (1)).

Imperial Oil 862B and presumably other closely related hydrocarbon fractions can be applied in the form of emulsions in low volume and with good leaf coverage. Calpuzos et al. (3) have noted fungistatic activity of an oil spray used against *Mycosphaerella musicola*. Imperial Oil 862B is fungistatic to rust infections when applied post-infection in greenhouse tests, but the delay in growth of rust infections (leaf rust of wheat and crown rust of oats) lasts for 3 or 4 days only and the final extent of growth of the rust infection is approximately that of the checks. In these tests there is definite evidence of protective fungicidal activity on the part of the oil.

The results of this paper have shown that it is possible to enhance the residual protective action of nickel salts with surfactants and other additives. They also provide evidence that the polybutene Indopol L-10, certain anionic (dodecylbenzene sodium sulfonate and the constituent of Atlox G-3300) and cationic (Hyamine 3500) surfactants and the oil Imperial Oil 862-B can be effective fungicides against the cereal rusts.

Literature Cited

1. BROWN, I. F., and H. D. SISLER. 1960 Mechanisms of fungitoxic action of N-dodecylguanidine acetate. Phytopathology 50: 830-839.
2. CALPOUZOS, L., T. THEIS, CARMEN M. RIVERA and C. COLBERG. 1959. Studies on the action of oil in the control of Mycosphaerella musicola on banana leaves. Phytopathology 49: 119-122.
3. CALPOUZOS, L., ALMA SANTIAGO, T. THEIS and C. COLBERG. 1960. Evidence of fungistatic action of petroleum oil against Mycosphaerella musicola inside banana leaves. Phytopathology 50: 865-866.
4. FISHER, R. W. 1959. Polybutenes. A promising control for powdery mildew. Plant Disease Reptr. 43: 878-879.
5. FORSYTH, F.R. and B. PE'PURSON. 1959. Chemical control of cereal rusts. IV. The influence of nickel compounds on wheat, oat, and sunflower rusts in the greenhouse. Phytopathology 49: 1-3.
6. FORSYTH, F.R. and B. PETURSON. 1960. Control of leaf and stem rust of wheat by zineb and inorganic nickel salts. Plant Disease Reptr. 44: 208-211.
7. KEIL, H. L., H. FHOHLICH and C. E. GLASSICK. 1958. Chemical control of cereal rusts. III. The influence of nickel compounds on rye leaf rust in the greenhouse. Phytopathology 48: 690-695.

PESTICIDE RESEARCH LABORATORY,
CANADA DEPARTMENT OF AGRICULTURE,
LONDON, ONT.