Detection of Antifungal Activity in Potato Tubers Field-Treated with Metalaxyl, a Systemic Fungicide

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ABSTRACT

Bhatia, S. K., and Young, R. J. 1983. Detection of antifungal activity in potato tubers field-treated with metalaxyl, a systemic fungicide. Plant Disease 67:1075-1079.

The systemic fungicide metalaxyl (Ridomil), when applied to soil or foliage, effectively controlled potato late blight. Tubers of the cultivar Katahdin were harvested from plants treated with metalaxyl and/or conventional fungicides and tested for antifungal activity. No evidence of fungal inhibition was observed in tubers sampled from plants treated with standard fungicides, but tubers sampled from some metalaxyl-treated plants showed completely inhibited growth and development of *Phytophthora infestans*, indicating an antifungal substance was present. Additional experiments confirmed that the antifungal property was uniformly distributed to all areas of the tuber, and the effect persisted beyond 110 days in storage both at 5 and 20 C. Tubers 10 cm in diameter and larger were more resistant to infection than smaller tubers. When metalaxyl was applied at 0.414 kg/ha or less, no antifungal activity was detected by this bioassay. Higher rates of metalaxyl (1.7 kg/ha) caused tubers to break dormancy earlier; sprouts developed sooner and more abundantly.

Late blight caused by *Phytophthora infestans* (Mont.) de Bary is a major factor limiting annual potato production in the high-rainfall northeastern region of the United States. Yields are reduced when the pathogen infects and destroys the foliage prematurely and tubers are destroyed when inoculum produced on the foliage is washed into the tuber zone.

Losses of 10% caused by tuber rot have been reported (3,5), but when late blight is severe, losses resulting from early killing of foliage and subsequent tuber rot in the field and storage are as great as 20% of the total crop grown in the Northeast (8).

Late blight has been controlled traditionally by applying protectant fungicides. Fungicides belonging to the copper, dithiocarbamate, and heterocyclic nitrogen groups have been used most frequently. Recent development and introduction of systemic compounds with fungicidal properties may offer the potato grower a new and more efficient weapon to combat this important disease.

Metalaxyl, a systemic fungicide, is an example of these compounds. Metalaxyl is specifically effective at low rates against plant-pathogenic Oomycetes (1,11,13,14) and has been used successfully to control late blight, downy mildews, and soilborne diseases incited by *Phytophthora* species (2,6,7,10,12).

Results from annual fungicide tests conducted in our laboratory showed a reduction of infected tubers whenever metalaxyl was used (R. J. Young, *unpublished*). Because control of *P. infestans* was also good in foliage when metalaxyl was used, there were at least two possible explanations for the reduced incidence of blight in the tubers. First, control of foliar blight resulted in an increased number of nonsporulating lesions, effectively reducing inoculum. Second, because metalaxyl has systemic fungicidal properties, it could accumulate in the physiologically active tubers at concentrations inhibitory to the pathogen.

This paper reports some of our findings resulting from the use of metalaxyl to control potato late blight. Our primary objective was to determine whether some substance accumulated in tubers at levels inhibitory to *P. infestans*. It was also of interest to determine whether treatment with metalaxyl caused other disturbances in the normal physiology of the tubers.

MATERIALS AND METHODS

Plant and pathogen. Disease-free seed tubers of cultivar Katahdin were used in these tests. Both the foliage and tubers of this cultivar are susceptible to all physiological races of *P. infestans*. Plants were spaced 25 cm apart in rows 90 cm apart. Four fungicides were evaluated in 17 treatments replicated six times. Each replicate consisted of four rows 15 m

Accepted for publication 1 April 1983.

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long, but only 9 m of the center two rows were used to collect yield and disease data. *P. infestans* race 1, 2, 3, 4 was used for the laboratory tests. The isolate was cultured on lima bean agar and incubated at 18 C in the dark.

Fungicides and fungicidal applications. Three standard fungicides, mancozeb, captafol, and chlorothalonil, and the systemic fungicide metalaxyl were used in the experiment. Standard fungicides were applied as sprays to the foliage at weekly intervals in a volume of water equal to about 938 L/ha at 250 psi. Metalaxyl was applied either as a foliar spray or as a soil treatment before final cultivation (hilling). Combination treatments of foliar and soil applications of metalaxyl also were examined. Initial fungicide applications were made during the second week of July and were continued on a 7-, 14-, or 21-day schedule, depending on the treatment, through the first week of September.

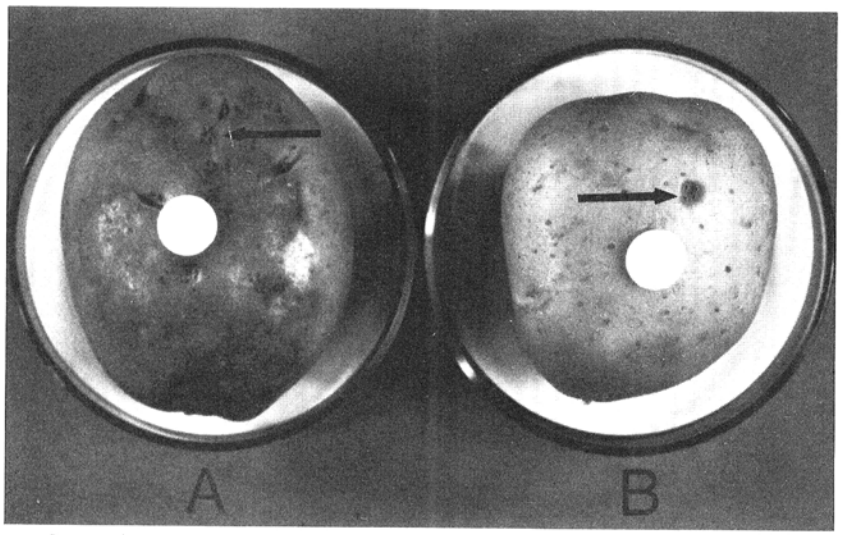

Fig. 1. Comparison of **(A)** untreated whole tubers and **(B)** metalaxyl-treated tubers of cultivar Katahdin inoculated with *Phytophthora infestans*. Arrow indicates point of inoculation.

Table 1. Evaluation of freshly harvested and stored Katahdin potato tubers from 17 fungicide treatments for an antifungal property inhibitory to the growth of *P. infestans*

Treatment no.	Treatment and formulation	Rate	Schedule	Tuber slice rating[a]	
				Test 1	Test 2[b]
1	Captafol 4F	2.3 L/ha	7 Days[c]	5.0	5.0
2	Chlorothalonil 6F	1.2 L/ha	7 Days	4.0	5.0
3	Mancozeb M-45 80WP	2.2 kg/ha	7 Days	4.0	5.0
4	Metalaxyl 5G	1.7 kg/ha	At hilling	1.0	2.0
5	Metalaxyl 2EC	0.21 kg/ha	14 Days	4.5	5.0
6	Metalaxyl 2EC	0.28 kg/ha	14 Days	4.0	4.5
7	Metalaxyl 2EC	0.28 kg/ha	21 Days	4.0	4.0
8	Metalaxyl 2EC	0.55 kg/ha	21 Days	1.5	3.0
9	Metalaxyl 5G +	1.7 kg/ha	At hilling	...	...
	metalaxyl 2EC	0.21 kg/ha	14 Days	1.0	2.0
10	Metalaxyl 2EC +	0.14 kg/ha	14 Days	...	...
	mancozeb M-45	1.33 kg/ha	14 Days	4.0	4.5
11	Control	None	...	5.0	5.0
12	Metalaxyl 2EC	0.414 kg/ha	Single application[d]	5.0	5.0
13	Metalaxyl 5G +	1.1 kg/ha	At hilling	1.0	2.0
	metalaxyl 2EC	0.21 kg/ha	14 Days	...	...
14	Metalaxyl 2EC	0.414 kg/ha	Single application[e]	5.0	5.0
15	Metalaxyl 2EC	0.414 kg/ha	Single application[f]	4.0	5.0
16	Metalaxyl 2EC	0.414 kg/ha	Single application[g]	5.0	5.0
17	Metalaxyl 5G +	1.7 kg/ha	At hilling	...	...
	metalaxyl 2EC	0.21 kg/ha	21 Days	1.0	1.5

[a] Tuber slices evaluated on a scale of 1 = resistant to 5 = susceptible.
[b] Test 1, freshly harvested; test 2, stored for 110 days at 5 C.
[c] Number of applications: 7-day interval, six or seven; 14-day interval, three or four; and 21-day interval, two.
[d] Single application at incipient infection.
[e] Single application at 10% infection.
[f] Single application at 25% infection.
[g] Single application at 40% infection.

At maturity, tubers were harvested and samples obtained from each treatment. Tubers were used for laboratory experiments within 30 days or stored at either 5 or 20 C. Tuber materials obtained from the field or from refrigerated storage were held for 24 hr at room temperature. They were then washed in tap water, rinsed in distilled water, and air-dried at room temperature for 1–2 hr. The tubers were then carefully surface-sterilized with 5% commercial Clorox for 10 min, rinsed with sterile distilled water, and air-dried.

Preparation of inoculum and tuber slice inoculation procedure. Ten-day-old cultures of *P. infestans* in petri dishes were flooded with 20 ml of precooled (4 C) sterile distilled water and shaken gently to dislodge sporangia. The sporangial suspension was then poured into a sterile petri dish and kept at 12 C for 2 hr to stimulate zoospore liberation. The inoculum was filtered and adjusted to a concentration of about 10,000 zoospores per milliliter. Ten-millimeter-diameter antibiotic assay disks were dipped into the inoculum and applied to the freshly cut surface of a tuber slice or to a puncture wound made in a whole tuber (Fig. 1). Each filter-paper disk absorbed a volume of about 0.01 ml. In one set of experiments, slices also were inoculated with mycelial disks taken from the margins of actively growing cultures of *P. infestans*. After inoculation, the tuber slices were incubated at 19 C for 7 days, then scored on a scale of 1 = no growth through 5 = extensive growth.

In experiments requiring tuber slices, 1-cm-thick slices were cut with a sharp knife from the crown, middle, and heel-end regions of the tubers. The slices were placed in 9-cm-diameter petri dishes containing a disk of previously moistened Whatman No. 2 filter paper. The papers were remoistened periodically with sterile distilled water to maintain high humidity. Slices were inoculated immediately to minimize the effect of the wound healing process associated with injured potato tubers.

RESULTS

Tests for antifungal activity were conducted on samples of 20 tubers from each treatment about 30 days after harvest and again after 110 days in storage. Tubers from plants treated with the three standard fungicides failed to demonstrate antifungal activity in either test (Table 1, Fig. 1). Tubers sampled from some metalaxyl treated plants also failed to show complete inhibition to the growth of *P. infestans* (treatments 1, 2, and 3 with 5, 6, 7, and 11, Table 1). Also, tubers from single applications of 0.414 kg/ha applied at various stages of defoliation failed to demonstrate antifungal activity (treatments 12, 14, 15, and 16, Table 1). Antifungal activity was demonstrated only in tubers sampled

from plants treated with metalaxyl at rates of 0.555 kg/ha or higher, either as a granular (G) or emulsifiable concentrate (EC) formulation (treatments 4, 8, 9, 13, and 17, Table 1 and Fig. 2). Supplementing the granular (5G) treatments (treatments 9, 13, and 17 with 4, Table 1) applied at hilling with metalaxyl 2EC at 0.21 and 0.28 kg/ha (treatments 9, 13, and 17, Table 1) on 14- and 21-day schedules failed to demonstrate any increase in inhibition. The level of activity in tubers from these treatments was not affected appreciably during the 110-day storage period, but activity appeared to decline in the 0.55 kg/ha EC treatment (treatment 8, Table 1) after 110 days in storage. There did not appear to be any synergistic effect on accumulation of antifungal activity by combining mancozeb M-45 and metalaxyl (treatment 10, Table 1).

Effect of storage temperature and tuber size on longevity of antifungal factor. Antifungal activity in tuber samples stored at 5 and 20 C as long as 110 days was assessed using the tuber slice technique (Table 2). Tuber slices from small, medium, and large tubers were used in this experiment. No appreciable change in tissue reaction occurred in the controls; they were completely susceptible at the beginning as well as the end of the test. In contrast, when metalaxyl was applied either as a soil treatment (treatment 4, Table 1) or as a combination soil/foliar treatment (treatment 17, Table 1), there were only slight changes in the level of inhibition observed during storage. This was true for tubers stored at either 5 or 20 C and for large and medium-sized tubers. In addition, the antifungal activity persisted for more than 110 days, regardless of storage conditions.

Tissue from small tubers was more extensively invaded in two of the three metalaxyl treatments (Table 2). Strong inhibition of *P. infestans* growth was noted after 30 days in tubers sampled from plants treated with metalaxyl 2EC at 0.55 kg/ha applied on a 21-day schedule. Inhibition was nearly complete in large and medium-sized tubers stored 30 days at 20 C. A similar level of activity was noted in slices from large tubers by 50 days of storage at 5 C. Thereafter, the residual inhibitory activity declined rapidly, regardless of temperature conditions or tuber size (treatment 8, Table 2).

Effect of antifungal activity on zoospore and mycelial inoculum. Slices from tubers stored at both 5 and 20 C for 110 days were inoculated with either zoospores or mycelial inoculum. Slices obtained from large, medium, and small tubers were tested.

Control slices showed complete susceptibility to either kind of inoculum, whereas slices from the combination soil/foliar treatment (treatment 17, Table 3) showed nearly complete inhibition to either type of inoculum. Growth of *P. infestans* on tissue slices obtained from large and medium tubers exposed to a soil application of metalaxyl 5G was inhibited, whereas growth on slices from small tubers was weak. Only slight differences between types of inoculum were observed. After 110 days in storage, tubers from the foliar treatment showed a greatly reduced level of activity, with small differences between inoculum types.

Distribution of antifungal activity within the tubers. Distribution of the antifungal activity within the tuber was examined by inoculating the cortical and medulary tissues of slices obtained from the crown, middle, and heel portions of the tubers. Under these test conditions, no growth of *P. infestans* was observed in

Table 2. Effects of temperature, storage period, and tuber size on inhibition of *P. infestans* (race 1, 2, 3, 4) to infect Katahdin potato tubers field-treated with metalaxyl

	Tuber slice ratings at various storage periods and temperatures[a]						
	30 Days	50 Days		80 Days		110 Days	
Treatment	20 C	5 C	20 C	5 C	20 C	5 C	20 C
Control (11)[b]							
Large[c]	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Medium	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Small	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Metalaxyl 5G, 1.7 kg/ha (4)							
Large	1.0	1.0	1.0	1.5	2.0	1.5	1.5
Medium	1.0	1.0	2.5	2.5	3.0	2.0	1.5
Small	3.5	4.0	4.0	3.0	3.5	3.0	4.5
Metalaxyl 2EC, 0.555 kg/ha (8)							
Large	1.0	1.0	3.0	4.0	4.5	4.0	4.0
Medium	1.0	3.0	3.0	4.5	4.0	3.5	3.5
Small	3.0	4.0	4.0	4.5	4.5	3.5	3.5
Metalaxyl 5G, 1.7 kg/ha + metalaxyl 2EC, 0.21 kg/ha (17)							
Large	1.0	1.0	1.0	1.0	1.5	1.5	1.5
Medium	1.0	1.0	1.0	1.5	1.5	1.5	1.5
Small	1.0	1.0	1.0	1.5	1.5	1.5	1.5

[a] Tuber slices evaluated on a scale of 1 = resistant to 5 = susceptible.
[b] Numbers in parentheses refer to treatments listed in Table 1.
[c] Tuber slices from large, medium, and small tubers.

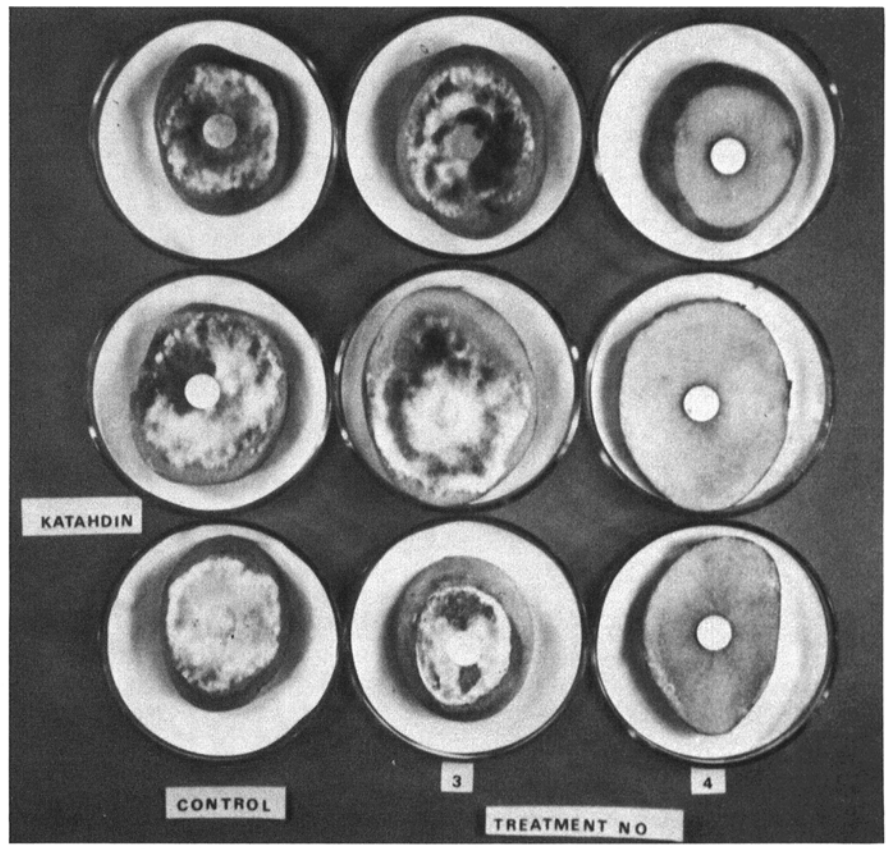

Fig. 2. Comparison of invasion by *Phytophthora infestans* in tuber slices from unsprayed (control) and (3) mancozeb- and (4) metalaxyl-sprayed plants.

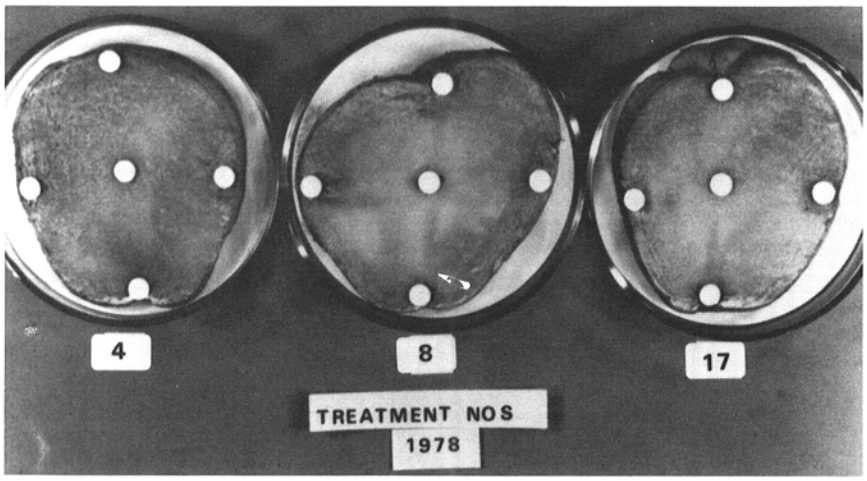

Fig. 3. Uniform distribution of an antifungal property in Katahdin tuber slices field-treated with metalaxyl and inoculated with *Phytophthora infestans*.

Table 3. Effect of an antifungal substance on two forms of inoculum of *P. infestans* on tuber slices from Katahdin potato tubers field-treated with metalaxyl and stored for 110 days at 5 and 20 C

	Tuber slices rating at 110 days storage[a]			
	Zoospore inoculation		Mycelial inoculation	
Treatment	5 C	20 C	5 C	20 C
Control (11)[b]				
Large slices[c]	5.0	5.0	5.0	5.0
Medium slices	5.0	5.0	5.0	5.0
Small slices	5.0	5.0	5.0	5.0
Metalaxyl 5G, 1.7 kg/ha at hilling (4)				
Large slices	1.5	1.5	1.5	2.0
Medium slices	2.0	1.5	2.0	2.0
Small slices	3.0	4.5	4.0	4.5
Metalaxyl 2EC, 0.555 kg/ha at 21 days (8)				
Large slices	4.0	4.0	4.0	4.5
Medium slices	3.5	3.5	4.0	4.5
Small slices	3.5	3.5	4.0	4.5
Metalaxyl 5G, 1.7 kg/ha at hilling + metalaxyl 2EC, 0.21 kg/ha at 21 days (17)				
Large slices	1.5	1.5	1.5	1.5
Medium slices	1.5	1.5	1.5	1.5
Small slices	1.5	1.5	1.5	1.5

[a] Tuber slices evaluated on a scale of 1 = resistant to 5 = susceptible.
[b] Numbers in parentheses refer to treatments listed in Table 1.
[c] Tuber slices obtained from large, medium, and small tubers.

Table 4. Detection of antifungal activity inhibitory to *P. infestans* in different regions of Katahdin potato tubers field-treated with metalaxyl

	Tuber slice rating[a]	
Treatment	Inoculation at cortex	Inoculation at medulla
Metalaxyl 5G, 1.7 kg/ha		
Slices from crown end	1.0	1.0
Slices from middle	1.0	1.0
Slices from heel end	1.0	1.0
Metalaxyl 2EC, 0.555 kg/ha		
Slices from crown end	1.5	1.5
Slices from middle	1.5	1.5
Slices from heel end	1.5	1.5
Metalaxyl 5G, 1.7 kg/ha + metalaxyl 2EC, 0.21 kg/ha		
Slices from crown end	1.0	1.0
Slices from middle	1.0	1.0
Slices from heel end	1.0	1.0
Control		
Slices from crown end	5.0	5.0
Slices from middle	5.0	5.0
Slices from heel end	5.0	5.0

[a] Tubers evaluated on a scale of 1 = resistant to 5 = susceptible.

either cortical or medulary tissues (Fig. 3, Table 4).

Effect of systemic fungicide on tuber dormancy. Effects of three selected metalaxyl treatments on tuber dormancy were examined (Table 5). Tuber samples stored at 5 C were examined after 110 and 150 days, and the lengths of sprouts were measured. Results clearly indicate that higher concentrations of metalaxyl increased sprouting and shortened the dormancy of tubers (Fig. 4). The average length of sprouts of tubers sampled from treatments where both soil and foliar applications were made was about five times that of sprouts of the control tubers. In treatments where plants received lower rates of metalaxyl, sprout length was similar to that of the control.

DISCUSSION

Tubers from plants receiving foliar applications of metalaxyl showed more reduced incidence of infection with *P. infestans* than did tubers from plants treated with either conventional fungicides or left unsprayed (R. J. Young, *unpublished*). Similar observations were made by Bruck et al (4) and Fry et al (9). Where conventional protectant fungicides were applied to foliage, effective control of foliar late blight was not necessarily related to the same level of control in the tubers. Tuber infection ranged from 6 to 9% in such treatments; however, metalaxyl treatments not only maintained a low level of foliar infection (<1%) but also maintained a similarly low level of tuber infection (R. J. Young, *unpublished*).

Laboratory assay of tubers sampled from metalaxyl-treated plants indicated that susceptible Katahdin tubers were resistant to infection by *P. infestans*. These results not only confirm earlier field observations but also provide a logical explanation for lower tuber rot. In treatments where metalaxyl was applied at 0.55 kg/ha or higher, tubers were resistant to infection. Tubers sampled from plants that received lower rates or fewer applications were either moderately resistant or fully susceptible, whereas all tubers from plants treated with conventional fungicides were susceptible.

These results indicate that an antifungal substance must have accumulated in the tubers, causing them to resist infection when inoculated with a virulent race of *P. infestans*. Furthermore, the antifungal substance persisted in tubers for more than 110 days when stored at either 5 or 20 C.

Tubers 10 cm in diameter and larger were more resistant than smaller tubers (6.35 cm). We suggest that larger tubers were probably initiated earlier in the growing season than the smaller ones and therefore were exposed to the systemic fungicide for a longer period or larger tubers could have behaved as highly active physiological sinks, thus accumu-

Table 5. Effect of field treatment with metalaxyl on dormancy of Katahdin potato tubers

Treatment	Average length of sprouts (cm) at various storage periods	
	110 days	150 days
Metalaxyl 5G, 1.7 kg/ha	0.11	0.46
Metalaxyl 2EC, 0.555 kg/ha	0.21	0.67
Metalaxyl 5G, 1.7 kg/ha + metalaxyl 2EC, 0.21 kg/ha	1.18	1.89
Control	0.11	0.40

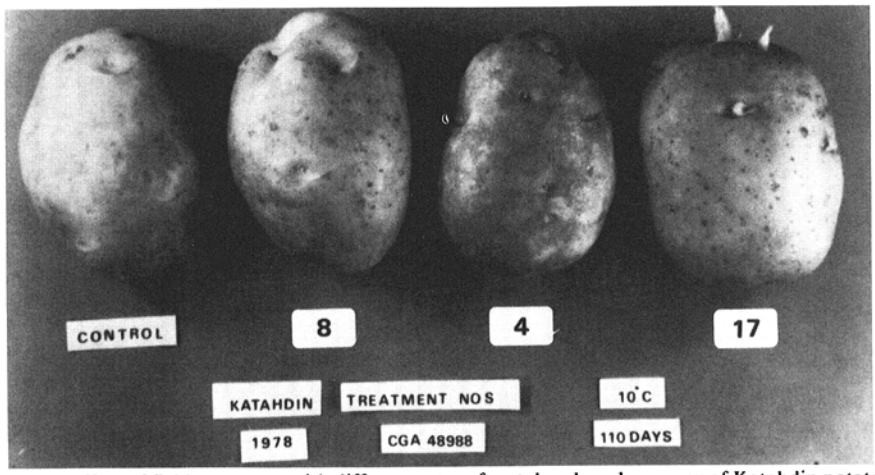

Fig. 4. Effect of field treatment with different rates of metalaxyl on dormancy of Katahdin potato tubers.

lating more photosynthate and resulting in accumulation of higher levels of the antifungal substance.

Accumulation of the antifungal substance appears to be related more to the total amount of systemic fungicide applied during the cropping season than to dosages alone (R. J. Young, *unpublished*). It is important to note that, regardless of the method of application, antifungal activity was demonstrated in the tubers. The sensitivity of the bioassay used to detect this activity indicated only that the concentration was high enough to prevent growth and development of *P. infestans* on the tuber slices. The assay did not indicate what relationship might exist between the total amount of systemic applied and the concentrations actually accumulated by the tuber tissue.

Our data show that if metalaxyl was applied at 0.414 kg/ha or less, no antifungal activity was detected by the bioassay. These tubers were assayed, at the earliest, 30 days after harvest or about 2.5 mo after the final treatment. Thus, there would have been ample time for lower concentrations, if present, to become ineffective in inhibiting *P. infestans*. Periodic testing of tubers at relatively short intervals after applying the systemic fungicide may determine when inhibitory or threshold levels of the antifungal substance occurred.

Neither mycelial nor zoospore inocula were able to infect slices taken from metalaxyl-treated tubers. Both kinds of inoculum were sensitive to the level of antifungal substance present in the tuber tissue. It was not determined whether penetration occurred, but it was clear that infection did not occur.

At higher rates, metalaxyl caused tubers to break dormancy earlier than untreated tubers. Lower rates had little to no effect on sprouting, whereas control of foliar and tuber blight was nearly as effective.

Except for Fry's (9) report, the literature is devoid of information with specific reference to the effect of metalaxyl on tuber blight. Its low, mamalian toxicity, combined with rapid absorption and prolonged effectiveness against *P. infestans*, makes metalaxyl a promising systemic fungicide for control of potato late blight. However, the accumulation of an antifungal substance in the tubers after treatment with metalaxyl should be noted.

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