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Impacts of Different Pentachloronitrobenzene (quintozene) Use Patterns on Severity of Damping-off of Ginseng (*Panax quinquefolius*) Caused by *Rhizoctonia solani*

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Abstract : In replicated field trials, the efficacy of pentachloronitrobenzene (PCNB; quintozene) for control of damping-off of ginseng seedlings was found to be affected by timing of application and formulation. Application at the time of seeding and prior to placement of straw mulch was found to provide the most consistent level of disease control. However, decline in plant stand during the four-year production cycle resulted in most treatments providing similar levels of plant populations at harvest. Soil residues of pentachloronitrobenzene were generally highest (1 μg PCNB/g soil) in those treatments that exhibited the highest levels of disease control in the seedling year. Straw contained high levels of quintozene after application. Beet seed assays with artificially-infested soils indicated that current use rates provide an amount of product suitable for high levels of disease control.

Key words : *Panax quinquefolius*, PCNB, quintozene, fungicide, disease, North American ginseng.

INTRODUCTION

Diseases are important constraints on the production of ginseng (*Panax quinquefolius*) in North America.[1,2] *Rhizoctonia solani* causes a damping-off disease as the young shoots emerge through the straw mulch in the spring and may also be associated with seed decay and crown rot of mature plants.[3] Various formulations of pentachloronitrobenzene (PCNB, quintozene) have been used to control this disease.[4] The persistence of this material after application[5,6] is a valuable characteristic when used in perennial crops; the product may be applied at planting and provide control of the target pathogen in subsequent growing seasons. In annual crops, however, use of this material can result in significant residues in roots and foliage at the end of the growing season.[5] Concerns relating to residues in ginseng roots at harvest and persistence in soil after harvest have led to questions regarding the most effective formulation and use pattern of this material. The objectives of this study were to assess the effects of different use patterns of pentachloronitrobenzene on control of damping-off of ginseng caused by *R. solani*.

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MATERIALS AND METHODS

1. Field plots

Three trials were established in consecutive years (1994, 1995 and 1996). Plots (2.5 m long×1.5 m wide) were laid out in three adjacent conventional ginseng beds, under plastic shade cloth. Soil type, foliar fungicide applications and general cultivation practices were as described previously.[7] Each plot was subdivided into two 1×1 m subplots, designed to receive pathogen inoculum either in the fall, after seeding, or the following spring. Inoculum consisted of *R. solani*-colonized ginseng root sections and was placed in the centre of each subplot. Fungicide treatments were applied as indicated below after seeding and inoculum addition. Fall fungicides were applied either before placing an oat (*Avena sativa*) straw mulch over the beds (pre straw) or after strawing (post straw). Spring fungicides were applied over straw. Rates used in all cases were those recommended by provincial authorities at the time of application. Spray boxes were used to reduce drift of product to adjacent plots. Subplots of any given plot received the identical fungicide treatments. Treatments and dates of treatment application are provided in Table 1.

Table 1. Treatments applied

Treatment[α]	Date of treatment application		
	Trial 1[§]	Trial 2[†]	Trial 3[‡]
1. Quintozene 75WP; @ 9Kg/ha; pre straw; fall	ND	Oct 27 1995	Oct 28 1996
2. Quintozene 75WP; @ 9Kg/ha; post straw; fall	Oct 14 1994	Oct 27 1995	Oct 28 1996
3. Plant Products 0-0-6+15%; @ 45Kg/ha; post straw; fall	Oct 14 1994	Oct 27 1995	Oct 28 1996
4. Quintozene 75WP; @ 9Kg/ha; pre straw; fall Quintozene 75WP; @ 9Kg/ha; post straw; spring	Oct 14 1994 May 2 1995	Oct 27 1995 May 3 1996	Oct 28 1996 May 7 1997
5. Plant Products 0-0-6+15%; @ 45Kg/ha; post straw; spring	May 2 1995	May 3 1996	May 7 1997
6. control	–	–	–

[α]Products supplied by Plant Products Co Ltd, Brampton ON.
[§]Trial 1: Treatment 1 was not included in trial 1(ND = not done). Inoculum was added to Fall-inoculum plots on 14 Oct 1994 and to Spring-inoculum plots on 2 May 1995. An additional application of quintozene (Nutri-Q 0-0-5 @ 135 kg product/ha) was made to all plots 18 April 1996. Nutri-Q 0-0-5 (Nutrite Inc, Elmira, ON) is a granular formulation of quintozene, similar to Plant Products 0-0-6, but contains only 5% quintozene. Plant Products 0-0-6 contains 15% quintozene.
[†]Trial 2: Inoculum was added to Fall-inoculum plots on 27 Nov 1995 and to Spring-inoculum plots on 1 May 1996. An additional application of quintozene (Nutri-Q 0-0-5 @ 135 kg/ha) was made to all plots 18 Nov 1996.
[‡]Trial 3: Inoculum was added to Fall-inoculum plots on 25 Nov 1995 and to Spring-inoculum plots on 5 May 1996. An additional application of quintozene was not made.

Table 2. Trial 1: End of season (August) stand counts

Treatment[¶]	Fall inoculum								Spring inoculum							
	Stand count (plants/m^2) years 1-4								Stand count (plants/m^2) years 1-4							
	Yr 1		Yr 2		Yr 3		Yr 4		Yr 1		Yr 2		Yr 3		Yr 4	
2	98.3	bc*	48	bc*	43.3	b*	33.3	ns*	179.3	b*	17.5	b*	9.5	ns*	14.5	ns*
3	186.8	a	110.3	ab	114.8	a	63.3	ns	226	a	89.3	a	35.7	ns	9.5	ns
4	213.7	a	118.2	a	95.8	ab	39.2	ns	224.7	a	73.2	ab	21.2	ns	13.2	ns
5	166.2	ab	83	abc	98.3	ab	51	ns	237.3	a	78.2	a	18.2	ns	10.8	ns
6	79.3	c	39.7	c	35.7	b	24.2	ns	172.5	b	13.3	b	6.7	ns	9.5	ns
P>F	0.0002		0.0019		0.0191		0.6982		0.0003		0.0023		0.4952		0.6517	
CV	25.2		39.6		47.4		75.4		9.53		51.6		112.3		87.1	

[¶]Trial 1 contained only treatments 2-6.
*means within a column followed by the same letter are not significantly different at P<0.01 (where the P>F value is less than 0.01), or P<0.05 (where the P>F value is equal to or greater than 0.01 but less than 0.05), using Tukey's studentized range test. ns-not significant.

In trials 1 and 2, the listed treatments were followed by an overall application of a granular formulation (Nutri-Q 0-0-5; Nutrite Inc., Elmira, ON) of pentachloronitrobenzene in either the fall of the seedling year or in the spring of the second year. No overall application was made in the third trial.

To prepare inoculum, freshly dug ginseng roots were cleaned and sliced into sections approximately 0.5 cm thick. Sections were placed in 500 mL erlenmeyer flasks (approx. 100 mL per flask) and flasks were then autoclaved (115°C) for 1 h on each of two consecutive days. After cooling to room temperature (RT), agar blocks from 7-10-day-old potato dextrose agar cultures of *R. solani* (isolate DRS 895; AG 2-1) were added to the flasks. Cultures were held at RT under ambient light conditions for approx. 1 mo before adding to field plots. Approx 5 g of colonized ginseng root was placed in the centre of each subplot. Dates of inoculum addition for each trial are listed in Table 1.

Radial extension of disease from the central inoculum point in each subplot was determined at intervals throughout each growing season. The extent of disease spread was marked for the south and west direction at the initial measurement using plastic markers. Markers were moved at each measurement if disease had spread and new measurements recorded. Ginseng stand counts for a 1.0 m^2 area subplot were also taken during each growing season. Positioning of the counting frame (1 m^2) was marked using ®Ringot fluorescent plot stakes in three corners at the initial count. The counting frame was relocated on these markers for each subsequent stand count. Soil samples were collected at intervals for soil quintozene residue

analyses, using HPLC. Selected samples of straw mulch were also analyzed for quintozene residue. Fall-inoculum and spring-inoculum data were analysed separately. Data were analysed using GLM (SAS; SAS Institute, Cary, NC) and Tukey=s test for studentized ranges.

2. Beet seed assays of fungicide efficacy

A modified beet seed assay[8] was used to assess effects of rates of pentachloronitrobenzene on growth of *R. solani* in soil. Wheat seed cultures[9] of *R. solani* were grown for 7-14 days under ambient laboratory conditions (20±2 C). Field soil was sieved then double-autoclaved at 115°C (1 h×2). After cooling, soil moisture was adjusted to 70% water holding capacity (WHC) with sterile water and, for each treatment, 200 g of autoclaved soil was transferred into a sterilized beaker. Colonized wheat seed was mixed into soil (1 g wheat seed culture/kg soil) and autoclaved beet seeds (1 g/200 g soil) were added. The soil was then mixed thoroughly and divided equally into four autoclaved glass petri plates so that each plate contained 50 g of soil (4 replicate-plates per treatment). Treatments consisted of soil prepared as described above then amended with one of six rates (0, 0.02, 0.2, 0.5, 0.9, and 1.4 mg a.i./cm^2 soil) of pentachloronitrobenzene (Plant Products 0-0-6; Plant Products Co Ltd, Brampton ON) by adding the product to the soil surface. The soil was dampened with a mister to bring the WHC of the soil to 80% and plates were held at 24±1 C for 2 days. Beet seeds then were recovered from the soil by sieving, washed for 20 minutes with cold tap water, and placed on a selective agar medium.[10]. Agar with seeds was held at 20±1 C for 1 day, then seed colonization was determined by assigning each seed to a colonization index[11]. Mean percent colonization for each treatment replication was calculated and used in statistical analyses. Data from two trials were combined prior to analysis. Data were arc-sine transformed and subjected to two-way analysis of variance (SigmaStat, SPSS Science Inc, Chicago IL), followed by mean separation using Tukey's Test. Non-linear regression analyses were performed (SigmaPlot, SPSS Science Inc, Chicago IL) on untransformed data.

RESULTS

1. Field trials

(1) Trial 1

Tables of data are attached (Tables 2, 3, 7, 8). In fall-inoculum control (no fungicide) subplots, massive disease outbreaks were evident in the seedling year and most plants failed to emerge through the straw mulch. This indicated that inoculum was virulent and that conditions for disease development were satisfactory (Tables 2, 3). Stand count data and disease radii data for fungicide-treated plots during the seedling year suggest that most of this disease occurred in early spring (April-May) rather than the fall. The effectiveness of the spring granular applications clearly showed this effect. Generally, granular treatments (Plant Products 0-0-6) were effective when applied over straw and the wettable powder (WP) treatments provided little control when applied over straw. When spring inoculum data are examined, it appears that fall and spring granular applications and fall pre-straw WP applications (treatments 3, 4, 5) are effective in controlling damping-off resulting from spring inoculum. Pre-

Table 3. Trial 1: End of season (August) disease radii

Treatment	Fall inoculum								Spring inoculum							
	Disease radius (m) years 1 to 4								Disease radius (m) years 1 to 4							
	Yr 1		Yr 2		Yr 3		Yr 4		Yr 1		Yr 2		Yr 3		Yr 4	
2	0.47	ab*	0.45	ab*	0.45	ab*	0.46	ab*	0.29	a*	0.57	ns*	0.5	ns*	0.5	ns*
3	0.3	bc	0.3	bc	0.31	b	0.31	c	0.19	b	0.37		0.4		0.5	
4	0.24	c	0.25	c	0.32	b	0.34	c	0.17	b	0.41		0.5		0.5	
5	0.32	bc	0.31	bc	0.32	b	0.37	bc	0.16	b	0.39		0.5		0.5	
6	0.56	a	0.52	a	0.5	a	0.5	a	0.33	a	0.56		0.5		0.5	
P>F	0.0004		0.0003		0.034		0.0285		0.0001		0.0513		0.2316		0.6656	
CV	24.2		21.1		25.2		20.2		19.4		25.8		12.4		7.5	

*means within a column followed by the same letter are not significantly different at P<0.01 (where the P>F value is less than 0.01), or P<0.05 (where the P>F value is equal to or greater than 0.01 but less than 0.05), using Tukey's studentized range test. ns-treatments not significantly different.

Table 4. Trial 2: End of season (August) stand counts

Treatment	Fall inoculum								Spring inoculum							
	Stand count (plants/m^2) years 1-4								Stand count (plants/m^2) years 1-4							
	Yr 1		Yr 2		Yr 3		Yr 4		Yr 1		Yr 2		Yr 3		Yr 4	
1	119.7	ns*	115.5	a*	68.3	a*	12.2	ns*	128.2	ns*	106	ns*	56.5	ns*	10.3	ns*
2	120	ns	95.8	ab	47.8	ab	5.2	ns	121.5	ns	106	ns	64.2	ns	12.2	ns
3	122	ns	120.2	a	57.5	ab	8.8	ns	142.5	ns	119.3	ns	57.5	ns	9.8	ns
4	128.7	ns	122.2	a	64.7	ab	4.8	ns	127.2	ns	100.2	ns	43.2	ns	6.8	ns
5	108.8	ns	106.8	ab	58.3	ab	2.2	ns	150.5	ns	117.3	ns	36.5	ns	8	ns
6	89.7	ns	63.7	b	22.7	b	2.8	ns	119.5	ns	93.8	ns	42.5	ns	4	ns
P>F	0.5371		0.0396		0.0062		0.1984		0.444		0.1174		0.2508		0.0932	
CV	27.1		26.8		38.1		86.6		26.4		27.3		53.5		76.8	

*means within a column followed by the same letter are not significantly different at P<0.01 (where the P>F value is less than 0.01), or P<0.05 (where the P>F value is equal to or greater than 0.01 but less than 0.05), using Tukey's studentized range test. ns-not significant.

Table 5. Trial 2: End of season (August) disease radii

Treatment	Fall inoculum								Spring inoculum							
	Disease radius (m) years 1 to 4								Disease radius (m) years 1 to 4							
	Yr 1		Yr 2		Yr 3		Yr 4		Yr 1		Yr 2		Yr 3		Yr 4	
1	0.17	c*	0.21	b*	0.23	b*	0.28	ns*	0.16	ns*	0.22	ns*	0.26	ns*	0.33	ns*
2	0.27	abc	0.3	ab	0.28	b	0.4	ns	0.23	ns	0.29	ns	0.34	ns	0.32	ns
3	0.21	bc	0.22	b	0.28	b	0.37	ns	0.25	ns	0.3	ns	0.37	ns	0.35	ns
4	0.18	c	0.23	b	0.32	ab	0.36	ns	0.22	ns	0.32	ns	0.36	ns	0.42	ns
5	0.33	ab	0.34	ab	0.36	ab	0.37	ns	0.16	ns	0.26	ns	0.39	ns	0.39	ns
6	0.38	a	0.43	a	0.44	a	0.44	ns	0.25	ns	0.29	ns	0.36	ns	0.36	ns
P>F	0.0006		0.0001		0.0056		0.2778		0.4112		0.2296		0.405		0.2411	
CV	27.1		22.1		21.1		23.5		34.7		32.2		27.9		27.4	

*means within a column followed by the same letter are not significantly different at P<0.01 (where the P>F value is less than 0.01), or P<0.05 (where the P>F value is equal to or greater than 0.01 but less than 0.05), using Tukey's studentized range test. ns-not significant.

Table 6. Residues of pentachloronitrobenzene in soil (μg/g soil)[†]

Treatment	Trial 1 (seeded Fall 1994)						Trial 2 (seeded Fall 1995)						Trial 3 (seeded Fall 1996)			
	Sample date:						Sample date:						Sample date:			
	Oct 95		Nov 96		Sep 98		Nov 96		Dec 97		Nov 96		Jul 97		Dec 97	
1	NA[§]		NA[§]		NA[§]		0.57	ab*	0.18	ns*	1.4	ab*	1.04	a*	0.21	ab*
2	1.06	c*	0.53	ns*	0	ns*	0.04	b	0	ns	0	b	0.01	b	0	b
3	3.4	b	1.02	ns	0.1	ns	0.03	b	0	ns	0.44	b	0.08	b	0	b
4	5.6	a	0.14	ns	0.1	ns	0.8	a	0	ns	3.28	a	0.96	a	0.38	a
5	0	c	0.38	ns	0.78	ns	0.07	b	0.13	ns	0	b	0.06	b	0	b
6	0	c	0.85	ns	0	ns	0	b	0	ns	0	b	0.04	b	0	b
P>F	0.0001		0.2936		0.4762		0.0012		0.4722		0.02		0.0001		0.0001	
CV	47.4		122.9		488.2		92.6		240		150.6		47.8		118.4	

[†]Analyses were carried out by Uniroyal (Elmira ON)
[§]NA-data not available.
*means within a column followed by the same letter are not significantly different at P<0.01 (where the P>F value is less than 0.01), or P<0.05 (where the P>F value is equal to or greater than 0.01 but less than 0.05), using Tukeys studentized range test. ns-not significant.

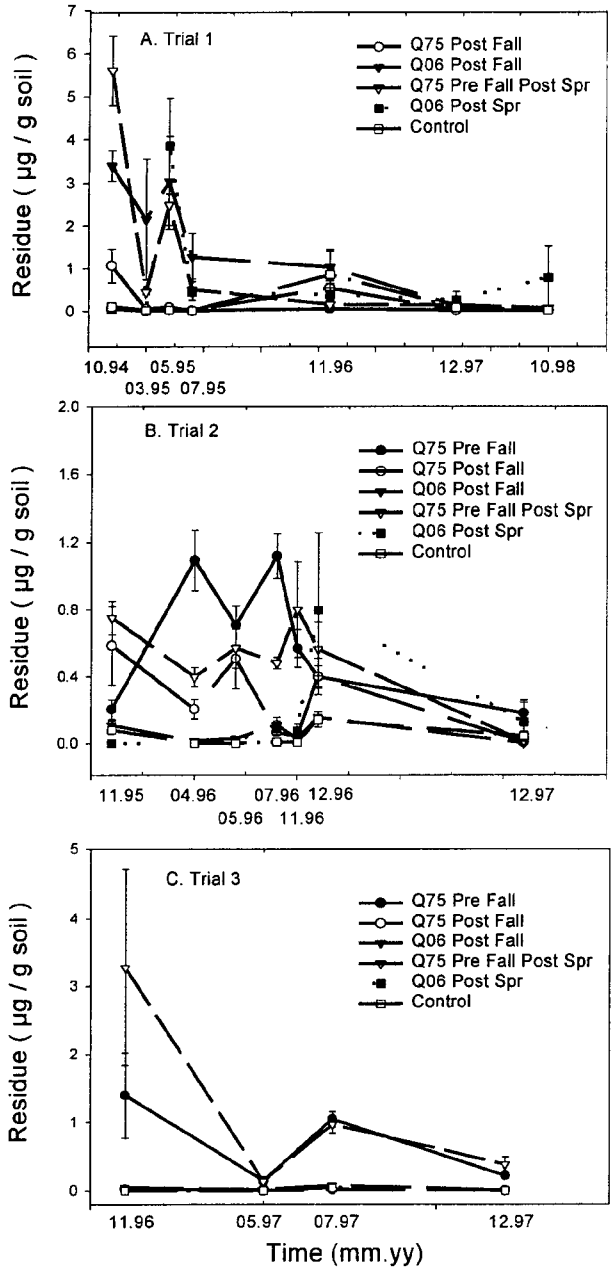

Fig. 1. Residues of pentachloronitrobenzene (Fg/g soil) in soil at various intervals after application. A. Trial 1, established in Fall 1994. B. Trial 2, established Fall 1995. C. Trial 3, established Fall 1996. Dates on which soil samples were collected are shown on the horizontal axis, where the first two digits represent the month and the last two digits represent the year. For each figure, treatment are: Q75 Pre Fall = treatment 1; Q75 Post Fall = treatment 2; Q06 Post Fall = treatment 3; Q75 Pre Fall Post Spr = treatment 4; Q06 Post Spr = treatment 5; Control = treatment 6.

straw WP plus spring WP was not superior to fall post-straw granular applications. Soil residue data were quite variable, as indicated by high CV values (Table 6). The overall granular application had a transient effect on soil residue levels (Fig. 1).

In the second year, significant differences occurred in both the fall and spring-inoculated subplots for stand count and in fall-inoculated plots for disease spread. The data show that the relative order of treatment differences observed at the end of the first growing season was maintained at the end of the second growing season for both fall and spring-inoculated subplots. A greater decline in stand counts and increase in disease area occurred in spring-inoculated subplots relative to the fall- inoculated subplots. An apparent decline in control plots, more severe in the spring-inoculated subplots, occurred despite an overall treatment with granular quintozene on April 18, 1996. In the third year, significant differences were found in the fall-inoculated subplots for stand count and disease spread. Differences between treatments were no longer apparent in the spring-inoculated plots. The relative order of treatment differences observed at the end of the first growing season were maintained at the end of the third growing season for both fall and spring-inoculated subplots. The data indicate an apparent continued decline in stand in the spring-inoculated subplots between year 2 and 3. An apparent stabilization in stand decline between year 2 and 3 was observed in the fall-inoculated subplots. In the fourth year, differences between treatments were maintained only for fall-inoculum plot disease radius. Here, pre-straw WP and granular applications continued to be superior to control treatments. Soil residue values were not significantly different at this time.

(2) Trial 2

Tables of data are attached (Tables 4, 5, 7, 8). Ginseng plant germination and emergence were very erratic in the spring of 1996 in this garden due to unfavourable weather. As a consequence, patterns of disease spread and plant emergence were not clear enough to evaluate until June and stand differences were not apparent in the first year of growth (Table 4). Damping-off radius was significantly (P=0.01) less in all fall-applied quintozene treatments in the fall-inoculated sub-plots, except for the post-straw WP treatment (Table 5). No significant treatments effects were observed for either ginseng plant stand or disease spread in the spring-inoculated subplots. Only a minor increase in average disease radii occurred over the growing season in all plots. Fall fungicide applications tended to result in higher soil residue levels (Table 6, Fig. 1B). In the second year, significant differences (P=0.05) were apparent among the fall-inoculated subplots for stand count and disease

spread. These differences were apparent as early as the first observations taken on May 28, 1997. All treated plots generally maintained the same values as at the end of the first growing season (1996). No differences were apparent for the spring-inoculated subplots. An overall increase in diseased area and decrease in stand counts compared to the previous season was observed in the spring inoculated subplots. At the end of the third year, only the pre-straw WP treatment remained superior to the control for stand count. Pre-straw and post-straw WP and post-straw fall granular treatments were superior to the control for disease radius. At the end of the fourth year, all treatment differences had disappeared.

(2) Trial 3

Disease development was poor and therefore treatment differences were rare. Stand and disease radii data are therefore not shown for this trial. Soil residue data are shown (Table 6).

2. Beet seed assays

Both trials provided similar results and data were combined. There was a significant treatment effect ($P<0.001$) with treatments 1 and 2 (0 and 0.02 mg ai/cm^2 soil) exhibiting markedly more colonization than all other treatments. Treatment 2, however, did have significantly less ($P=0.028$) colonization than treatment 1. Treatments 3 to 6 (0.2 to 1.4 mg ai/cm^2 soil) were not significantly different from each other. The relationship between colonization of beet seeds in infested soil and concentration of fungicide was best shown with an exponential decay equation (Fig. 2).

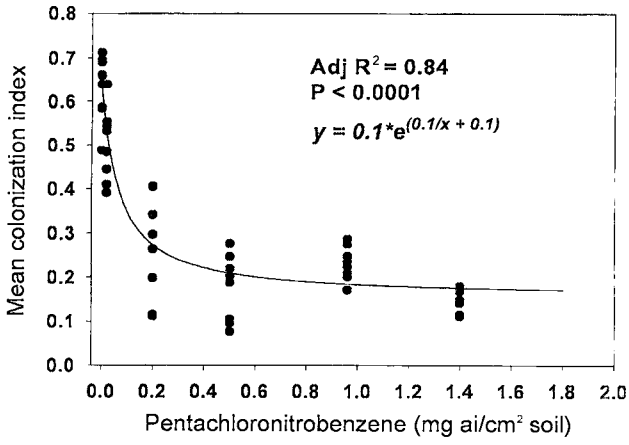

Fig. 2. Colonization of beet seed by *Rhizoctonia solani* in soils amended with different rates of pentachloronitrobenzene (mg ai/cm^2 soil). Data points represent combined data from two trials (n=8). The curve shown is an exponential decay curve.

DISCUSSION

Generally, fall-inoculum plots were more useful than spring-inoculum plots in comparing treatments. *R. solani* prefers cool, moist growing conditions[12] and the disease may develop slowly over the winter months and in the early spring prior to plant emergence. A unknown number of seeds were likely colonized and killed prior to seed germination. The addition of inoculum to plots was generally successful in generating disease epidemics and also enabled small plots to be used to compare products for efficacy. Where disease intensity was severe enough to allow for treatment comparisons, fall pre-straw applications provided the most consistent results. In trials, large declines in plant stand had occurred in all plots by the end of the fourth year, irrespective of treatment and irrespective of the level of damping-off in the seedling year. Thus differences among treatments tended to disappear over time. In part, this is a reflection of plot size: the extent damping-off is not measured beyond the plot boundary. Thus there is a maximum amount of damping-off that can occur in a given plot. As stand declines, plant populations in plots where *R. solani* is well-controlled are likely to fall to levels similar to those where significant damping-off occurred in the seedling year. Reasons for this general stand decline are unknown but may be related to root infections by *Cylindrocarpon destructans* or *Botrytis cinerea*, or perhaps to other factors. The source of *R. solani* in commercial plantings of ginseng is unknown but this pathogen has been isolated from diseased stratified seed (Reeleder, unpublished data). Infested seed may therefore be a source of inoculum in commercial ginseng production. Poor disease development in trial 3 may have been due to unusually hot, dry weather during the first three years of this trial, or to a loss in inoculum virulence. During all three trials, spring weather tended to be unusually hot and dry. This may account for the relatively poor development of disease in spring-inoculated subplots in these trials.

Soil residue data generally reflected the amount of disease control obtained. High levels of disease control appeared to be consistent with soil residues during the growing season of at least 1 μg PCNB/g soil (ppm). Recommended product rates result in applications equal to approximately 0.1 mg PCNB/cm^2 soil. Data from the beet seed colonization trial indicate that the amounts of PCNB deposited onto soil when these rates are used are sufficient to suppress *R. solani* and likely are close to the minimum amount required for high levels of disease control.

In all trials and all treatments, soil residues declined over time. Data over the four-yr production cycle were collected for the first trial only, but trends were similar in the remaining trials. Based on these data, it seems likely that detectable residues will be present in soil up to four years after application. To investigate the possible role of the straw mulch as a reservoir of PCNB, straw samples were collected from plots of treatments 4-6 on12 Nov 1996 from trials 1 and 2. One set of samples was collected prior to the overall quintozene application, the other subsequent to the overall application. Mean values for treatments 4, 5, and 6 prior to the overall application were 10.3 (±3.60), 7.2 (±2.48), and 0.4 (±0.05), respectively. Mean values for treatments 4, 5, and 6 subsequent to the overall application were 36.6 (±16.45), 20.8 (±6.31), and 25.4 (±16.62), respectively. These data suggest that residues on straw following application can be significant. The fate of this reservoir of quintozene is not known; some of the material may eventually be deposited in soil as a result of leaching events or straw degradation. In these studies, only the known active ingredient was determined in analyses. Metabolites formed as pentachloronitrobenzene degrades[5] were not monitored.

In summary, products containing pentachloronitrobenzene were shown to be useful in controlling damping-off of seedlings but performance depends in part upon the timing of application and the formulation used. Residues in soil are persistent and detectable for several years after application. Thus, not only may ginseng be affected by use of this material but subsequent crops may also be exposed to significant residues of pentachloronitrobenzene. Alternatives to this quintozene products are now becoming available to the ginseng industry.[13] Azoxystrobin and similar products appear to be promising new materials for control of this disease.

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