Registry No. M1, 119209-27-7; M2, 83792-61-4; M3, 626-43-7; vinclozolin, 50471-44-8.

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Persistence of the Fungicide Vinclozolin on Pea Leaves under Laboratory Conditions

Sunny Y. Szeto,* Nick E. Burlinson, James E. Rahe, and Peter C. Oloffs[1]

When pea leaflets were treated with Ronilan 50 WP or an acetone solution of vinclozolin under laboratory conditions, the fungicide persisted 21–46 days. Its persistence was higher with Ronilan 50 WP, a commercial formulation of vinclozolin, than with an acetone solution. However, most of the Ronilan deposits were easily dislodged by rinsing with water, indicating that Ronilan was susceptible to weathering. The dissipation of vinclozolin on leaves was linear, and the calculated half-life was 33.1 days for Ronilan and 13.4 days for the acetone solution. Translocation of vinclozolin was not detected in pea plants after its application to one of the leaflets. None of the hydrolytic degradation products, namely 2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methyl-3-butenoic acid (M1), 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide (M2), and 3,5-dichloroaniline, were detected in the treated plants. However, vinclozolin, M1, and M2 were detected in leaves of pea and bean grown in nutrient solutions containing either vinclozolin or its degradation product M1.

Vinclozolin [3-(3,5-dichlorophenyl)-5-methyl-5-vinyloxazolidine-2,4-dione] is marketed by BASF AG as a protectant fungicide under the trade name Ronilan. It is effective in the control of diseases caused by *Botrytis* spp., *Sclerotinia* spp., and *Monilinia* spp. in grapes, fruits, vegetables, ornamentals, hops, rapeseed, and turfgrass (Spencer, 1982). This fungicide is widely used in Europe for controlling fungal diseases; and it is registered in the United States, but not in Canada.

Vinclozolin is known to undergo hydrolysis. Three degradation products, namely 2-[[(3,5-dichlorophenyl)-carbamoyl]oxy]-2-methyl-3-butenoic acid (M1), 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide (M2), and 3,5-dichloroaniline (M3), have been isolated from hydrolysis and identified by us (Figure 1) (unpublished data); and the kinetics of hydrolysis at various pHs and temperatures have been determined (Melkebeke et al., 1986; Szeto et al., 1989). According to Clark (1983) both M1 and M2 were noninhibitory in vitro against mycelial growth of *Botrytis cinerea*, indicating that they have no antifungal activity. M3 is a chlorinated aromatic amine that may be toxic to higher animals, and its possible formation is important to environmental toxicologists. Therefore, it is important to know whether these degradation products are formed in plants after application of vinclozolin.

This paper reports the findings of our studies under laboratory conditions on the persistence of vinclozolin and Ronilan 50 WP in garden pea, *Pisum sativum* L., and on translocation in garden pea and red kidney bean, *Phaseolus vulgaris* L., grown in nutrient solutions containing either vinclozolin or M1.

EXPERIMENTAL SECTION

Preparation of Vinclozolin and Its Hydrolytic Degradation Products. Vinclozolin, 2-[[(3,5-dichlorophenyl)carbamoyl]]oxy-2-methyl-3-butenoic acid, and 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide were obtained from BASF Aktiengessellschaft, and their purities were respectively, 99%, 98%, and 96%. 3,5-Dichloroaniline (98%) was obtained from the Laboratory Services Division of Agriculture Canada in Ottawa. Ronilan 50 WP was provided by BASF Canada Inc.

Growing Plants. Garden pea, *P. sativum* (cv. Improved Laxton's Progress), were seeded in square pots (10 cm × 10 cm × 9 cm) and grown in a greenhouse. Twelve days after seeding, the plants were used to study the persistence of vinclozolin and Ronilan 50 WP. For the

Agriculture Canada Research Station, 6660 N.W. Marine Drive, Vancouver, B.C., Canada V6T 1X2 (S.Y.S.), Department of Chemistry, University of British Columbia, Vancouver, B.C., Canada V6T 1Y6 (N.E.B.), and Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6 (J.E.R., P.C.O.).

[1] Deceased, June 19, 1987.

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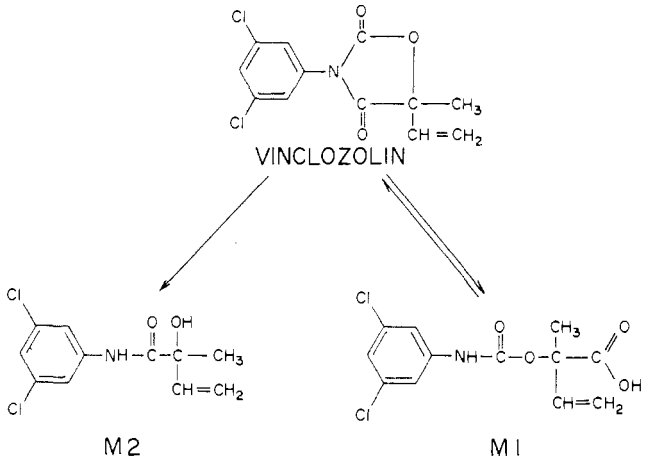

Figure 1. Degradation pathway proposed by Szeto et al. (1989) for the hydrolysis of vinclozolin leading reversibly to the formation of M1 and to M2.

translocation study, garden pea and red kidney bean, *P. vulgaris* (cv. Red Kidney), were germinated in a Petri dish by covering the seeds with moistened paper towels and 7-day-old seedlings were used for the experiment.

Treatment of Plants. For the persistence study, a solution of vinclozolin at 2500 μg/mL was prepared in acetone and a similar solution of Ronilan 50 WP containing 2500 μg/mL of vinclozolin was prepared by suspending 0.5 g of the formulation in 100 mL of water. After thorough mixing, four 5-μL aliquots of the Ronilan suspension were diluted individually with 2 mL of methanol and the actual concentration of vinclozolin was determined by HPLC. The mean concentration of vinclozolin was 2.60 μg/mL, which was comparable to the theoretical concentration of 2.5 μg/mL. The standard deviation among the four aliquots was 0.21 μg/mL. Twelve-day-old pea plants, each with only one pair of leaflets, were used to study the persistence of vinclozolin. Fifteen 5-μL aliquots of the treatment solution were applied to one of the paired leaflets in each plant. All treated plants were maintained in an environmental chamber in which the temperature varied from 24 to 27 °C and the RH from 40% to 70%. The photoperiod was 12 h each of light and darkness. At various intervals after treatment, both the treated and untreated leaflets were removed from the plant. The surface of the treated leaflet was rinsed with 10 mL of water, which was applied dropwise with a disposable Pasteur pipet. Both the treated and untreated leaflets and the rinsing were analyzed for vinclozolin and its degradation products M1, M2, and M3.

For the translocation study 7-day-old seedlings of garden pea and red kidney bean were grown in nutrient solutions initially containing 50 μg/mL of either vinclozolin or M1 at 22 °C in a laboratory. The nutrient solution was prepared by dissolving 0.35 g of Soiless Feed fertilizer (15–15–18) obtained from the Plant Products Co., Ltd. (Bramalea, Ontario, Canada L6T 1G1), in 1 L of distilled water fortified with either vinclozolin or M1 by adding an appropriate volume of the stock solution at 2500 μg/mL in acetone to give a final concentration of 50 μg/mL. During the study, the growth media were replenished with the unfortified nutrient solution whenever necessary. At various intervals after treatment the leaflets and the nutrient solutions were analyzed to determine the concentrations of vinclozolin and its degradation products.

Analysis of Rinsings. Leaflet rinsings were acidified to approximately pH 1.0 with a few drops of 2.5% H_3PO_4 and then extracted three times with 10 mL of glass-distilled dichloromethane in a 125-mL separatory funnel. The combined extracts were dried with acidified anhydrous Na_2SO_4 and concentrated just to dryness in a flash evaporator at 38 °C, and the residues were dissolved in 1 mL of methanol for HPLC analysis. The acidified anhydrous Na_2SO_4 was prepared by washing anhydrous Na_2SO_4 with glass-distilled acetone saturated with concentrated H_2SO_4 and then drying at 120 °C in an oven overnight.

Analysis of Leaflets. Leaflets of pea and bean were extracted in a Polytron homogenizer with 50 mL of glass-distilled acetone for 1 min. Ten grams of acidified anhydrous Na_2SO_4 was added to the mixture for extraction. The extracts were filtered through a Buchner funnel lined with Whatman GF/A glass fiber filter paper. The filter cake was extracted once more with 25 mL of glass-distilled acetone, and the combined extracts were evaporated just to dryness in a flash evaporator at 38 °C. The residues were dissolved in 5 mL of 1:1 (v/v) mixture of dichloromethane and cyclohexane for cleanup by gel filtration column chromatography.

A Pharmacia column, Model SR 25 (45 cm × 2.5 cm (i.d.)), was packed with Bio-Beads S-X12. The beads were swelled in a 1:1 (v/v) mixture of dichloromethane and cyclohexane overnight before packing the column. An Eldex Model B-100-S high-pressure pump was used for solvent delivery, and a Valco Model C6PX sample injection valve equipped with a 5-mL injection loop was used to inject samples onto the column. After introduction of a sample, the column was eluted with the 1:1 (v/v) mixture of dichloromethane and cyclohexane. Fraction 1, consisting of the first 68 mL, was discarded, and fraction 2, consisting of the next 150 mL, was collected. Fraction 2 contained the vinclozolin and its degradation products.

In order to isolate M1, fraction 2 was extracted three times with 25 mL each of 0.1 M Na_2HPO_4 solution. The combined aqueous extracts containing M1 were back-extracted with 25 mL of dichloromethane. After acidification to approximately pH 1.0 with 2.5% H_3PO_4, the combined aqueous extracts were extracted three times with 25 mL each of dichloromethane to isolate M1. The combined dichloromethane extracts were dried with acidified anhydrous Na_2SO_4 and then evaporated just to dryness in a flash evaporator at 38 °C. The residues of M1 were dissolved in 1 mL of methanol for HPLC analysis.

The combined organic extracts containing vinclozolin, M2, and M3 were dried with acidified anhydrous Na_2SO_4 and then evaporated just to dryness in a flash evaporator at 38 °C. The residues were dissolved in 1 mL of glass-distilled dichloromethane and analyzed by GC–MS.

HPLC Analysis. Determination of vinclozolin, M1, M2, and M3 by HPLC was performed with a Varian Model 5000 high-pressure liquid chromatograph equipped with a Hewlett-Packard Model 1040A high-speed spectrophotometric detector. The operating parameters were as follows: column, Varian Micro Pak MCH-10, 30 cm × 4 mm (i.d.); mobile solvent system, 72% methanol and 28% 0.05 M phosphate buffer of pH 3.3, isocratic at 1 mL/min; UV detector wavelength, 212 ± 2 nm. Detector response was calibrated with reference standards of vinclozolin, M1, M2, and M3 in methanol. Quantification was based on the average peak areas of these external standards, which were injected before and after each sample. Under the chromatographic conditions described, the absolute retention times were 5.04, 6.43, 7.25, and 8.86 min for M1, M3, M2, and vinclozolin, respectively.

Determination by GC–MS. Vinclozolin, M2, and M3 were detected and quantified with a Hewlett-Packard Model 5890 gas chromatograph equipped with a Model

Table I. Recoveries of Vinclozolin and Its Degradation Products from Water

compound	% recovery ($X \pm$ SD, $n = 4$) 10.0 ppm	1.0 ppm
vinclozolin	97.5 ± 1.6	96.6 ± 2.3
M1	92.5 ± 2.2	86.9 ± 2.0
M2	95.1 ± 2.2	93.0 ± 2.4
M3	90.2 ± 3.2	90.8 ± 3.0

Table II. Recoveries of Vinclozolin and Its Degradation Products from Pea Leaflets

compound	% recovery ($X \pm$ SD, $n = 4$) 10.0 ppm	1.0 ppm	0.2 ppm
vinclozolin	96.3 ± 2.0	95.2 ± 2.8	92.9 ± 2.6
M1	85.0 ± 3.5	81.4 ± 2.7	73.8 ± 3.6
M2	95.2 ± 2.9	94.9 ± 3.4	93.5 ± 2.8
M3	86.9 ± 3.7	81.0 ± 6.4	80.5 ± 5.2

5970 mass selective detector. The operating parameters were as follows: column, DB-17 fused silica capillary column, 30 m × 0.253 mm (i.d.); column head pressure, 140 kPa with a total flow of 40 mL of helium/min; column temperature, programmed from 70 to 225 °C at 20 °C/min with the final temperature held for 15 min; injector temperature, 225 °C; transfer line temperature, 250 °C; injection, splitless. Under the chromatographic conditions described, the absolute retention times were 7.04, 10.37, and 11.57 min for M3, vinclozolin, and M2, respectively. Qualitative and quantitative determinations were based on selected ion monitoring. Four specific ions characteristic of each compound were selected for monitoring, and they were as follows: M3, 90, 126, 161, and 163 amu; vinclozolin, 198, 212, 285, and 287 amu; M2, 71, 72, 161, and 163 amu. Detector response was calibrated each time with reference standards of vinclozolin, M2, and M3. Quantification was based on the average peak areas of these external standards, which were injected before and after each sample.

Evaluation of Methods. Stock solutions containing vinclozolin, M1, M2, and M3 each at 1000, 100, and 20 μg/mL were prepared in glass-distilled acetone. Of the 1000 μg/mL stock solution, 1 mL was diluted with water in a 100-mL volumetric flask to give a fortification level of 10 ppm for each compound; a 1 ppm fortification level in water was obtained similarly, using the 100 μg/mL stock solution. Four replicates of 10 mL each of the fortified aqueous solutions were directly analyzed by HPLC as described to determine recoveries of the compounds from water.

Ten grams of leaflets was fortified with vinclozolin, M1, M2, and M3 at concentration levels of 10, 1, and 0.2 ppm by adding 0.1 mL of the appropriate stock solutions in acetone. One hour after fortification four replicates at each concentration level were extracted, prepared, and analyzed as described to determine the recovery of each compound.

RESULTS AND DISCUSSION

Efficiency of Analytical Methods. The recoveries of vinclozolin, M1, M2, and 3,5-dichloroaniline from water and from pea leaflets are given in Tables I and II. The mean recoveries from water at 10 and 1 ppm ranged from 86.9% to 97.5% whereas the mean recoveries from pea leaflets at 0.2, 1.0, and 10.0 ppm ranged from 73.8% to 96.3%. The method had a high degree of precision at each fortification level as indicated by the small standard deviation.

Persistence on Pea Leaflets. Pea leaflets were treated with Ronilan 50 WP at 187.5 μg of vinclozolin/leaflet and analyzed for vinclozolin and its hydrolytic degradation products at various intervals after treatment. Since the recommended application rate is 1.0 kg of AI/ha in 400 L of water for fungal disease control with Ronilan 50 WP, the treatment rate used in this study is realistic. The results are given in Figure 2. There was 169 μg of vinclozolin present in the treated leaflet 2 h after treatment, which was approximately 90% of the theoretical amount of 187.5 μg. Four days after treatment the washings of the treated leaflets were also analyzed. Vinclozolin was the only compound detected in the washings of treated leaflets and in the treated leaflets themselves. The amount recovered from the washings of treated leaflets accounted for more than 70% of the total amounts (washing + leaflet), and the amounts in the washings in percent of the total increased with time. After 46 days the amounts of vinclozolin recovered from the washings accounted for approximately 94% of the total, and vinclozolin which remained adsorbed on the treated leaflet accounted for approximately 6% of the total. Linearity of the relationship between the total amounts of vinclozolin recovered from the treated leaflets and time was observed ($n = 12$, $r = -0.994$*, significant at $p = 0.05$), and there was no statistically significant deviation from linearity. Based on the linear regression equation amount = $-2.575t + 169.7$, the calculated half-life of vinclozolin on Ronilan-treated leaflets was 33.1 days.

The amounts of vinclozolin on pea leaflets treated with an acetone solution at the same rate at various, shorter

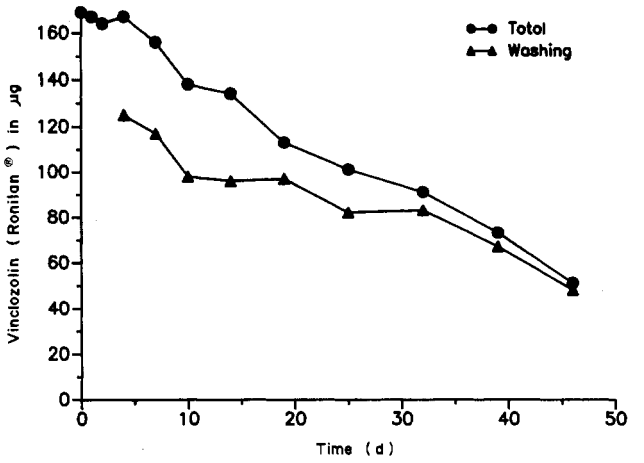

Figure 2. Persistence of vinclozolin on pea leaflets under laboratory conditions after application of aqueous solution of Ronilan 50 WP at 187.5 μg of AI/leaflet.

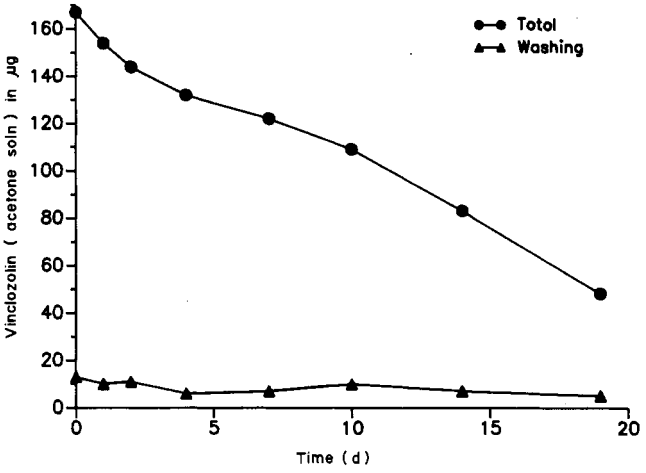

Figure 3. Persistence of vinclozolin on pea leaflets under laboratory conditions after application of an acetone solution at 187.5 μg/leaflet.

intervals after treatment are shown in Figure 3. There was 167 μg of vinclozolin present in the treated leaflet 2 h after treatment, which was comparable with the initial deposits on the leaflet treated with Ronilan (Figure 2). There was significant difference in the persistence of vinclozolin between the two treatments. Little vinclozolin was removed by washing with water from leaflets treated with the acetone solution. The amounts of vinclozolin present in the washings accounted for only 5–10% of the total (washing + leaflet). Linearity of the relationship between the total amounts of vinclozolin recovered from the treated leaflets and time was again observed ($n = 8$, $r = -0.993$*, significant at $p = 0.05$), and there was no statistically significant deviation from linearity. Based on the linear regression equation amount = $-5.750t + 160.8$, the calculated half-life of vinclozolin on the treated leaflets was 13.4 days.

The persistence of vinclozolin on treated leaflets was clearly influenced by the formulations used for application. Vinclozolin applied via the commercial formulation, Ronilan 50 WP, was much more persistent than from the acetone solution. The rate of disappearance of vinclozolin from Ronilan was about 45% of that from the acetone solution, so that its half-life was about 2.5 times longer. In Ronilan the active ingredients are impregnated in the inert solid support, thus reducing dissipation by physical means such as volatilization. In comparison, the acetone solution of vinclozolin spread with the solvent over the surface of the leaflets. Therefore, loss of the active ingredient by volatilization and codistillation during transpiration likely occurred. Biological degradation may be another important factor contributing to the lower persistence of vinclozolin from the acetone solution. Using acetone as the carrier likely promoted penetration of the active ingredient into the waxy surfaces of the pea leaflets, as evidenced by the fact that less than 10% of vinclozolin was removed by water whereas 90% or more was extracted by organic solvent (Figure 3). In contrast 75–95% of the active ingredient was removed by water from the Ronilan-treated leaflets (Figure 2). Vinclozolin, which penetrated through the waxy surfaces, would be subjected to possible biological degradation by the cells; reduced persistence, as was demonstrated, would be expected.

The fact that most of the active ingredient of Ronilan 50 WP was easily removed by rinsing with water has significant influence on its efficacy. In order to prevent infection, vinclozolin, a protectant fungicide, is applied to the surface of foliage or fruit to form a barrier between the host and the inoculum. Best protection is achieved by covering the surface with the protectant fungicide during the entire infection season. However, weathering gradually reduces the amount of residue on the surface, and eventually it drops below the threshold of effectiveness. In addition plant growth further dilutes the protectant. Therefore, protective treatment must be repeated at certain time intervals. Rain as one of the important factors of weathering removes deposits from treated plants by mechanical erosion. Since the deposits of Ronilan were easily removed by rinsing with water, it is evident that this protectant fungicide would be susceptible to weathering factors such as rain and dew. To increase the effectiveness of Ronilan, spreaders and stickers may be added to improve its resistance to weathering, or the frequency and timing of treatments may be synchronized in such a way as to compensate for loss of protectants due to weathering and growth.

The persistence of vinclozolin under field conditions is lower than that under laboratory conditions as reported here, likely because of weathering factors (rain, radiation). Zenon-Roland and Gilles (1978) reported that when vinclozolin was applied at rates from 125 to 175 g of AI/ha to strawberry plants, its residues on fruits were proportional to the application rate. The half-life varied from year to year. It was 12–13 days in one year and 22 days in the following year; both values were shorter than the half-life of 33 days reported here with Ronilan. Much shorter half-lives were reported on grapes. Del Re et al. (1980) determined the rate of dissipation after the last of seven treatments with vinclozolin at 700–1400 g of AI/ha and reported a half-life of 7.5–9 days. A still shorter half-life was reported by Gennari et al. (1985), also on grapes; depending on the application rate the calculated half-life ranged from 1.2 to 4.9 days.

Vinclozolin was not detected in any of the untreated pea leaflets after application of Ronilan or the acetone solution of vinclozolin to one of the leaflets, indicating that it was nonsystemic under the conditions of this study, i.e., not translocated from the treated to the untreated leaflets. In order to confirm this nonsystemic property, its possible movement was monitored 38 days after the Ronilan treatment and 21 days after the acetone solution treatment. All untreated plant parts including stems and roots were extracted and analyzed. Vinclozolin was not detected at the limit of detection of 0.1 ppm (fresh weight). We conclude that vinclozolin is nonsystemic when applied to leaves.

The hydrolytic degradation products, M1, M2, and M3, were not detected in either the treated or the untreated parts of the pea plant including root and stem, indicating that these compounds were not formed as residues in pea plants when vinclozolin was topically applied to the leaf surface. Similar observations were reported for 3-(3′,5′-dichlorophenyl)-5,5-dimethyloxazolidine-2,4-dione (DDOD), a methyl analogue of vinclozolin (Sumida et al., 1973). Bean plants (*P. vulgaris*) were injected with [^{14}C]DDOD at the stem. After 14 days, no degradation product was detected in the treated plants.

Translocation from Nutrient Solution. When pea plants were grown in nutrient solution fortified with 50 ppm of vinclozolin, both the parent compound and one of its hydrolysis product M2 were detected in the leaflets after 18 and 26 days (Figure 4a). Most of the vinclozolin in the nutrient solution was converted to M1 because of hydrolysis (Szeto et al., 1989). There were 2.34 ppm of vinclozolin in the nutrient solution after 18 days and none after 26 days (Figure 4b). Another contributing factor for the low concentration of vinclozolin in the nutrient solution was its low water solubility, resulting in loss through volatilization and codistillation. Considering the high concentration of M1 and low concentration of vinclozolin present in the nutrient solution, the vinclozolin detected in pea leaflets may have resulted from the translocation of M1 rather than vinclozolin. This hypothesis was confirmed by our findings in pea grown in nutrient solution fortified with 50 ppm of M1 (Figure 5a,b). The reversible ring closure from M1 to vinclozolin occurred in the nutrient solution as reported by Szeto et al. (1989). M1 appeared to be stable in the nutrient solution, and there was still 46.1 ppm after 26 days (Figure 5b). Most of the residues detected in pea leaflets were vinclozolin. There were 21.4 ppm after 18 days and 28.9 ppm after 26 days. M1 and M2 were also detected in the leaflets but at much lower concentration (Figure 5a). Our results clearly demonstrate that M1 was translocated from the nutrient solution to the pea leaflets and converted to vinclozolin during this process.

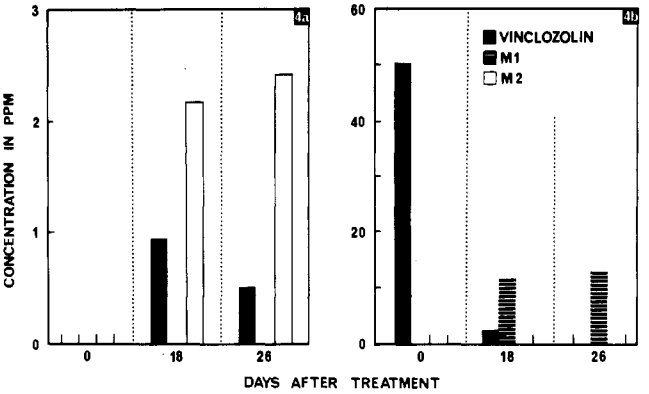

Figure 4. Distribution of vinclozolin, M1, and M2 in (a) pea leaflets grown in vinclozolin-treated nutrient solution for 26 days and (b) vinclozolin-treated nutrient solution.

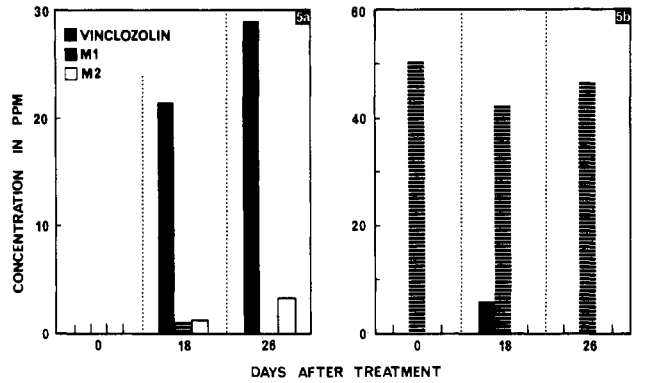

Figure 5. Distribution of vinclozolin, M1, and M2 in (a) pea leaflets grown in M1-treated nutrient solution for 26 days and (b) M1-treated nutrient solution.

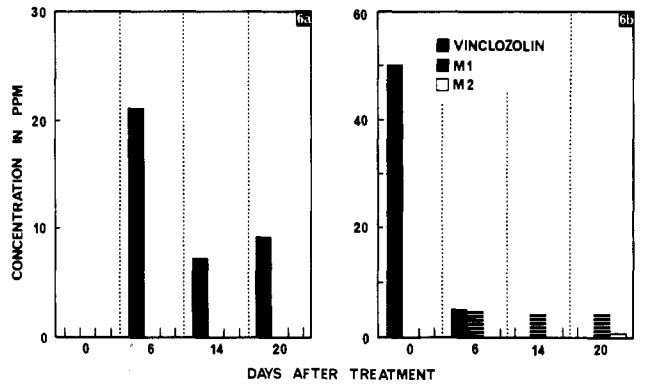

Figure 6. Distribution of vinclozolin, M1, and M2 in (a) bean leaflets grown in vinclozolin-treated nutrient solution for 20 days and (b) vinclozolin-treated nutrient solution.

Similar studies were conducted with bean plants (*P. vulgaris*) to confirm these findings. Translocation in bean of vinclozolin, or its degradation product M1, was similar to pea except that much higher concentrations of vinclozolin were present in the leaflets of beans than of peas (Figure 6a,b and 7a,b). Vinclozolin was never detected in the M1-treated nutrient solution used for beans, but there were 160 ppm in the leaflets after 6 days, 182 ppm after 14 days, and 66 ppm after 20 days (Figure 7a). In comparison, the highest concentration of vinclozolin was 8.63 ppm in the leaflets of bean plants grown in nutrient solution fortified with 50 ppm of vinclozolin for 20 days (Figure 6a). These results further confirm our findings that M1 is readily translocated from roots to leaves and converted to vinclozolin during this process. Regardless of the type of treatment, M1 and M2 were detected as minor

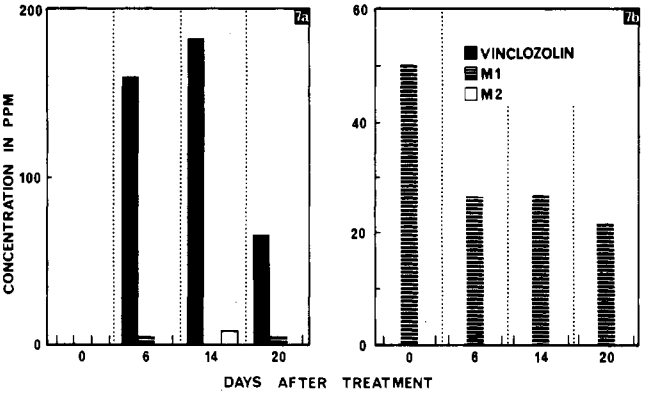

Figure 7. Distribution of vinclozolin, M1, and M2 in (a) bean leaflets grown in M1-treated nutrient solution for 20 days and (b) M1-treated nutrient solution.

components of residue in the leaflets of treated plants and M3 (3,5-dichloroaniline) was never detected in our studies at the limit of detection of 0.1 ppm. The highest concentrations of M1 and M2 were 2.8 and 8.62 ppm, respectively, detected in leaflets of bean plants grown in M1-fortified nutrient solution (Figure 7a).

M1 contains a carboxylic group that could ionize readily in the nutrient soluton and depending upon the pH the molecule could undergo recyclization to yield vinclozolin (Szeto et al., 1989). M1 could easily be absorbed through the roots, translocated through the symplast of the sieve elements where the pH is approximately 8, thus favoring its stability. In the apoplast around the phloem where the pH is approximately 5 (Jacob and Neuman, 1983) vinclozolin would be formed by recyclization of M1. Because of its extremely low water solubility (Spencer, 1982) vinclozolin would then be stored in the leaves. This phenomenon is of great interest to the plant pathologists as M1 would have the potential to be used as a systemic fungicide when this chemical is absorbed through the root. At present, very little is known about the bioactivity of M1, which is a stable hydrolytic product of vinclozolin. Therefore, more research on its bioactivity would be necessary.

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Isolation and Identification of Microorganisms for the Degradation of Dicamba

James P. Krueger, Robert G. Butz, Yousef H. Atallah, and Douglas J. Cork*

Eight species of soil bacteria from five genera capable of utilizing Dicamba (3,6-dichloro-2-methoxybenzoic acid) as a sole carbon source were isolated and identified. All strains were obtained by enrichment from soil and water with a long history of Dicamba exposure. Strains DI-6, DI-7, and DI-8 removed an average of 97% of the Dicamba from liquid culture in 30 h, with up to 80% of the substrate carbon being converted to CO_2. Degradative activity in liquid culture was observed over a wide pH range. Strains DI-6, DI-7, and DI-8 were able to remove 98% of the Dicamba after 21 days from soil treated with 3.4 μg/g of Dicamba, as compared to 34% from uninoculated soil. The primary soil metabolite of Dicamba, 3,6-dichlorosalicylic acid, was greatly reduced in the inoculated soil with an increase in CO_2 evolution. Thus, these three strains were able to rapidly reduce soil Dicamba concentrations and more completely degrade Dicamba as evidenced by higher rates of mineralization.

The compound 3,6-dichloro-2-methoxybenzoic acid, hereafter referred to as Dicamba, is used as a pre- and postemergent herbicide for the control of annual and perennial broadleaf weeds and several grassy weeds. Dicamba is similar in herbicidal action to phenoxyalkanoic acid herbicides such as (2,4-dichlorophenoxy)acetic acid (2,4-D) but belongs to the class known as the benzoics. Dicamba is chemically stable, and there is considerable evidence suggesting that the degradation of Dicamba, in aerobic soils and water, is biologically mediated (Harger, 1975; Smith, 1973, 1974; Smith and Cullimore, 1975; Scifres et al., 1973). Aerobic soil degradation studies have demonstrated that Dicamba is metabolized to CO_2, with 3,6-dichlorosalicylate being the major metabolite identified (Harger, 1975; Smith, 1973). However, the biological factors involved in the metabolism of Dicamba have not been determined. There are no reports in the literature on the metabolism of Dicamba by a pure culture of microorganisms. The bacterial metabolism of chlorinated benzoates is determined by the number and position of chlorines on the aromatic nucleus (Horvath, 1971). Increased bacterial growth and increased O_2 consumption have been reported when soil organisms capable of growth on *o*-anisate were grown on Dicamba in the presence of *o*-anisate (Ferrer et al., 1985). However, no analytical data on the disappearance of Dicamba were presented.

Organisms capable of degrading Dicamba and/or its 3,5-isomer (3,5-dichloro-2-methoxybenzoic acid) may be useful for facilitating the rapid dissipation of both isomers from the environment (Krueger, 1984). Microbial degradation of Dicamba in the rhizosphere of crops has the potential for protecting susceptible crop species from Dicamba applied for weed control purposes. Dicamba degrading organisms would provide a source of genetic material that might be utilized for the development of Dicamba-resistant higher plants, including crop species (Krueger, 1984).

This study describes the isolation and identification of pure cultures of bacteria capable of utilizing Dicamba as a sole carbon source. Removal of Dicamba from liquid culture over a wide pH range and removal from soil are also demonstrated.

MATERIALS AND METHODS

Chemicals and Microbial Medium. Authentic reference standards used as carbon sources and used for the identification of degradation products had a purity of 98% or greater. [^{14}C]Dicamba (U-phenyl-^{14}C, 11.5 mCi/mmol, radiochemical purity greater than 98%) was synthesized by Pathfinder Labs Inc. To increase solubility, stock solutions of all chemicals used as carbon sources were prepared by titration with NaOH to pH 7.0. All chemical stock solutions were filter sterilized through 0.2-μm Teflon filters before being added to sterile media. All other chemicals were reagent grade or better, and all solvents were glass-distilled quality.

Reduced chlorine medium (pH 7.0) contained the following (in grams/liter): $K_2HPO_4 \cdot 3H_2O$, 1.826; KH_2PO_4, 0.87; $(NH_4)_2HPO_4$, 0.66; $MgSO_4$, 0.097; $MnSO_4 \cdot H_2O$, 0.025; $FeSO_4 \cdot 7H_2O$, 0.005; $CaSO_4$, 0.001. The medium contained less than the detectable level of chlorine (1 ppm) as determined by the method of Bergmann and Sanik (1957). The filter-sterilized carbon source was added after autoclaving. Solid agar Petri plates of this medium were prepared by the addition of 1.5% agar.

Organisms. Soil and water samples were obtained from the storm water retention ponds at the Dicamba manufacturing plant in Beaumont, TX. Microbial strains used for screening are described in Table I.

Microbial Screening Experiments. Microorganisms with known degradative functions (Table I) were inoculated into 50-mL reduced chlorine medium containing 100 μg/mL [^{14}C]Dicamba (58 000 dpm/mL, final volume 50 mL), 3,5-dichloro-2-methoxybenzoic acid, 3,6-dichlorosalicylate, or 3,5-dichlorosalicylate as a sole carbon source. All culture bottles (125-mL amber serum bottles) were

Biology Department, Illinois Institute of Technology, Chicago, Illinois 60616 (D.J.C.), and Sandoz Crop Protection Corporation, 1300 East Touhy Avenue, Des Plaines, Illinois 60018 (J.P.K., R.G.B., Y.H.A.).

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