Soil Biol. Biochem. Vol. 19, No. 4, pp. 451–457, 1987
Printed in Great Britain.

0038-0717/87 $3.00 + 0.00

MICROBIAL CYANIDE PRODUCTION IN THE RHIZOSPHERE IN RELATION TO POTATO YIELD REDUCTION AND *PSEUDOMONAS* SPP-MEDIATED PLANT GROWTH-STIMULATION

ALBERT W. BAKKER and BOB SCHIPPERS
Willie Commelin Scholten Phytopathological Laboratory, Department of Plant Pathology, State University Utrecht and University of Amsterdam, Javalaan 20, 3742 CP Baarn, The Netherlands

(*Accepted* 1 *December* 1986)

Summary—Inhibition of root cell energy metabolism is suggested to be responsible for potato yield reductions in short potato-rotation soils. Hydrogen cyanide is the microbial metabolite possibly involved in inhibition of energy metabolism. This is supported by the following observations: (1) approximately 50% of potato rhizosphere pseudomonads was shown to produce cyanide *in vitro*; (2) 5 μM HCN inhibited cytochrome oxidase respiration by at least 40% in intact potato roots *in vitro*; (3) cyanide production *in vitro* by *Pseudomonas* sp. isolate WCS361 depended on the Fe^{3+} concentration of the medium. Growth promoting fluorescent *Pseudomonas* spp isolates WCS374 and WCS358 did not produce cyanide *in vitro*.

A hypothesis, that potato plant growth is depressed in short potato rotation soils by the microbial production of cyanide in the rhizosphere is discussed. In such soils, bacteria producing specific siderophores increase growth by competing with cyanide-producing organisms for Fe^{3+}.

INTRODUCTION

Long-term rotational experiments at the Experimental Farm "De Schreef" and the Research Station for Arable Farming and Field Production of vegetables (PAGV) in the Netherlands showed that potato tuber yields decrease with increasing potato cropping frequency (short rotation effect) (Hoekstra, 1981; Lamers, 1981; Schippers *et al.*, 1985).

Analysis of the cause of these yield reductions points to an as yet unknown microbial factor in soils frequently cropped to potato (short potato-rotation soils). Incidences of diseases caused by regular soilborne potato pathogens such as *Rhizoctonia solani* Kühn, *Verticillium dahliae* Kleb., *Streptomyces* spp and *Erwinia carotovora* subsp. *carotovora* (Jones) Bergey *et al.* were not sufficiently high in short potato rotation soils to completely account for the yield reductions observed. Availability of N, P and K did not differ significantly between the different rotation soils and differences in soil structure did not seem to be involved (Hoekstra, 1981; Lamers, 1981; Schippers *et al.*, 1985; Scholte *et al.*, 1985).

A bioassay has been developed (P. A. H. M. Bakker *et al.*, 1987) to study effects of short potato-rotation soils on potato plant development. By using this bioassay, root development of potato stem cuttings in short potato rotation soil was shown to be inhibited within 8 days, compared to that in long rotation soil. Disease symptoms such as discolourations of the roots, lesions, or impaired root hair formation, however, were not observed. Potato yield reductions in short potato rotations are therefore thought to be due to impaired root functioning caused by harmful microorganisms (HMO), rather than by the direct damage caused by regular pathogens. Uptake of nutrients by plant roots is an energy (ATP) demanding process (Ayers, 1984). The production of ATP, mediated by cytochrome oxidase respiration, can be inhibited by cyanide. This inhibition causes electrons, released by oxidation of NADH in potato mitochondria, to follow the "alternative cyanide resistant respiratory pathway" to oxygen. Much energy is thereby lost as heat instead of being stored in ATP (Lambers, 1980) (Fig. 1). This raises the possibility that cyanide produced by rhizosphere microorganisms is involved in the inhibition of potato root functioning in soils frequently cropped to potato.

Cyanide is a secondary metabolite of several microorganisms. It can be produced directly from glycine and from cyanogenic glycosides (Knowles, 1976) both of which have been demonstrated in root exudates (Rovira and Davey, 1974; Curl and Truelove, 1985).

The possible role of cyanide in the inhibition of root development of potato stem cuttings and in potato yield reductions in short potato-rotation soil was therefore studied by analyzing: (a) the presence of cyanide-producing microorganisms in the rhizosphere in short and long rotation soils; (b) cyanide production in the rhizosphere of potato plants in soil frequently cropped to potatoes; (c) sensitivity of potato root cytochrome oxidase respiration to cyanide.

Potato yield reductions and inhibition of root development of potato stem cuttings in short potato rotation soil could be strongly reduced by seed tuber or root treatments with selected "plant growth-stimulating" fluorescent pseudomonads. This has been ascribed to siderophore-mediated competition for Fe^{3+} between the "plant growth-stimulating" pseudomonads and rhizosphere microorganisms harmful to plant growth (Geels and Schippers,

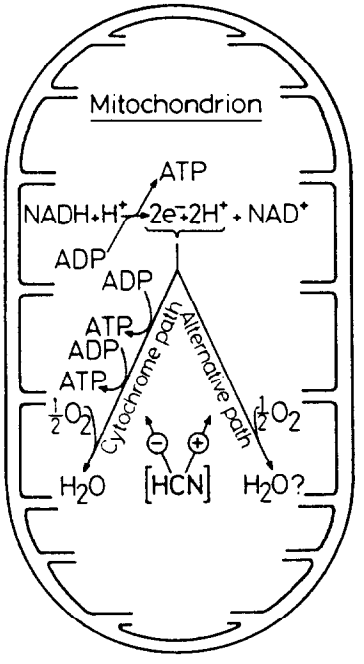

Fig. 1. The effect of cyanide on the efficiency of ATP production in plant mitochondria.

1983a, c; P. A. H. M. Bakker *et al.*, 1987). We therefore examined the effects of iron availability on cyanide production by rhizosphere microorganisms.

Initial results have been presented by Schippers *et al.* (1986).

MATERIALS AND METHODS

Screening of rhizosphere bacteria for cyanide production

Roots were taken from potato or wheat plants grown in field soil in pots in the greenhouse. Approximately 5 g fresh root material from each root system was thoroughly shaken for 20 min at 4°C in 100 ml sterile 0.1% proteose peptone solution in an Erlenmeyer flask containing quartz particles. Diluted suspensions were plated on tryptic soy agar (TSA), containing 3 g tryptic soy broth (Oxoid) l^{-1}, supplemented with 100 mg cycloheximide l^{-1}, and on King's medium B (KB) (King *et al.*, 1954) supplemented with 100 mg cycloheximide, 50 mg ampicillin and 12.5 mg chloramphenicol l^{-1} (KB^+). Colonies were counted after 48 h at 20°C. Ninety-eight distinct colonies on TSA and 50 on KB^+ were selected at random and transferred to TSA- and KB-media supplemented with 4.4 g glycine l^{-1} to screen for cyanide production.

Rhizosphere pseudomonads were isolated on KB^+ as described, from 8 potato and 8 wheat plants grown for 4 weeks in the greenhouse in field soil. The field soil was collected from short (1:1) and long (1:6) potato rotation soils, 10 weeks before harvest. Soil moisture was kept at 50% field capacity. Thirty *Pseudomonas* spp isolates were selected at random per plant and screened for cyanide production, to determine the proportion of rhizosphere pseudomonads producing cyanide.

Single isolates were streaked onto either TSA or KB supplemented with 4.4 g glycine l^{-1} to screen for cyanide production. Thereafter, the Petri dishes were inverted. A piece of filter paper impregnated with 0.5% picric acid (yellow) and 2.0% sodium carbonate was placed in the lid of each Petri dish. The Petri dishes were sealed with parafilm and held at 20°C for 96 h. Discolouration of the filter paper to orange-brown after incubation indicates microbial production of cyanide (Castric, 1975). To ascertain if discolouration was actually due to cyanide production, filter papers were impregnated with 1.0 M NaOH instead of picric acid and sodium carbonate. Cyanide trapped in NaOH solution was detected using the isonicotinic acid–barbituric acid method of Nagashima and Ozawa (1981).

The "plant growth-stimulating" *Pseudomonas fluorescens* isolate WCS374 and *Pseudomonas putida* isolate WCS358 (Geels and Schippers, 1983b) were also tested *in vitro* for their ability to produce cyanide. These isolates were also examined for their ability to antagonize five random selected HCN-producing *Pseudomonas* spp isolates on King's medium B, using the method of Geels and Schippers (1983b).

Detection of cyanide in the plant–soil system

Cyanide production in the plant–soil system was studied with five potato plants in a PVC pot containing 8 l soil from a field continuously cropped to potatoes. Potato plants were obtained from stem cuttings rooted in vermiculite for 10 days. A perforated tube system was used to suck air continuously out of the test soil (approx. 10 l h^{-1}). This air was led through a 5.0 M NaOH solution to trap HCN. The NaOH solution was sampled every 48 h, for 3 weeks. The isonicotinic acid–barbituric acid method was used to detect trapped cyanide. The water potential of the soil was kept constant at pF 2. Environmental conditions were similar to those described by P. A. H. M. Bakker *et al.* (1987). The experiment was repeated three times.

Sensitivity of cytochrome oxidase respiration of potato roots to cyanide

Stem cuttings of potato plants were rooted in vermiculite in pots in a controlled environment chamber (P. A. H. M. Bakker *et al.*, 1987). Vermiculite was moistened with nutrient solution containing l^{-1}: 3.2 ml 1 M KNO_3, 2.0 ml phosphate buffer pH 7.4 (24.3 g KH_2PO_4 and 30.7 g K_2HPO_4 l^{-1}, pH adjusted with NaOH and HCl), 1.0 ml solution containing 26.7 g $MgSO_4 \cdot 7H_2O$ and 5.0 g $MnSO_4 \cdot H_2O$ l^{-1}, and 1.2 ml of a micronutrient solution, which contained l^{-1}: 7.5 g H_3BO_3, 1.25 g $ZnSO_4 \cdot 7H_2O$, 0.25 g $CuSO_4$ and 0.25 g $Na_2MoO_4 \cdot 2H_2O$, pH adjusted to 5.0 with HCl. After 2 weeks, plants were removed from the vermiculite and used for root respiration experiments. Before respiration measurements started, plants were placed with their roots in aerated nutrient solution (20°C) of the same composition as that added to vermiculite, but without micronutrients. Roots were excised from the sprouts 5 min before respiration measurements started. O_2 uptake by in-

tact roots was measured polarographically with a Yellow Springs Instruments model 53 O_2 monitor (Lambers *et al.*, 1981). The temperature was kept at 20°C during respiration measurements. Three intact excised root systems were used per measurement. O_2 consumption of intact roots was linear for at least 25 min or until the O_2 concentration declined below 40% of air saturation. Salicylhydroxamic acid (SHAM) was used to inhibit the cyanide-resistant alternative respiration (Lambers *et al.*, 1981). SHAM was dissolved in the nutrient solution to a final concentration of 25 mM, and the pH was adjusted to 7.4 with 5.0 M NaOH. Effects of SHAM and cyanide on root respiration rate stabilized within 5 min after addition of the inhibitor. For measuring the effects of SHAM on root respiration, the nutrient solution was completely replaced by nutrient solution containing SHAM. Cyanide was added as KCN dissolved in 0.1 M NaOH in volumes of 0.25 ml. Measurements of effects of 5 μM KCN, 2.5 μM KCN and 1 μM KCN were repeated four, two and two times respectively.

Effect of Fe^{3+} concentration on microbial cyanide production

A lyophilized storage culture of fluorescent *Pseudomonas* sp. isolate WCS361 was seeded on King's medium B and grown for 2 days at 27 C. A suspension of this culture in sterile 0.1 M $MgSO_4$ was used as inoculum. A modification of the synthetic medium described by Castric (1975) was used containing: 20 mM L-glutamic acid; 10 mM glycine; 10 mM L-methionine; 2 mM $MgSO_4$; 5 mM NaH_2PO_4; 5 mM K_2HPO_4 and 100 mM Tris(hydroxymethyl)aminomethane. Traces of iron were removed from this medium by mixing it with 8-hydroxyquinolin. To do this 0.5 g 8-hydroxyquinolin dissolved in 5 ml 96% ethanol was added to 1 l medium. After mixing for at least 1 h on a magnetic stirrer at maximum speed, the complexed iron and remaining 8-hydroxyquinolin were extracted from the medium with chloroform (Meyer and Abdallah, 1978). The pH of the medium was adjusted to 7.0 with NaOH. Erlenmeyer flasks (750 ml) containing 150 ml medium per flask were sealed with rubber stoppers fitted with an air-inlet containing an air filter, an air-outlet and a sampling–inoculating device. This apparatus was sterilized for 30 min at 120°C to remove traces of chloroform. After sterilization, iron was added as heat-sterilized 10 mM $FeCl_3$. A possible effect of chloride addition was checked by addition of 0.3 mmol NaCl l^{-1} medium instead of $FeCl_3$. Five Erlenmeyer flasks with media were then shaken simultaneously in a water bath (20°C; 100 strokes min^{-1}). The air-outlet was connected with a cyanide trap containing 10 ml 2 M NaOH. Air flow was 1.5 l $flask^{-1}$ h^{-1}. The media were inoculated with cells suspended in 0.5 ml 0.1 M $MgSO_4$ and incubated for 5 days. The A_{600} of the media did not differ measurably before and after inoculation. At the end of the experiment, inoculum and samples from shake cultures were tested for their ability to produce cyanide on KB plates supplemented with glycine. A sample of the culture was supplemented with NaOH to a final concentration of 1 M and centrifuged. The cell-free supernatants were used for cyanide determination. Cyanide concentrations of supernatants and of the cyanide traps were measured using the isonicotinic acid–barbituric acid method.

Cell yield was determined at the end of the experiment by measuring the A_{600} of the shake cultures and the dry weight of cells of culture samples. The experiment, with three replicates for each $FeCl_3$-concentration, was repeated three times.

The effectiveness of the NaOH cyanide trap was checked by leading the air which had already passed through the trap, into a second trap. Hydrogen cyanide was determined in both traps.

RESULTS

Cyanide production by rhizosphere bacteria

From a total of 98 bacteria isolated from the rhizosphere of wheat or potato on TSA, four could produce cyanide. These four isolates grew well on the *Pseudomonas* spp-selective KB^+ medium. Twenty eight out of 50 isolates obtained on KB^+ produced cyanide on KD + glycine.

Cyanide-producing microorganisms were abundant in the rhizosphere of both potato and wheat and in both short and long potato rotation soil. The proportion of pseudomonads isolated from wheat or potato rhizosphere that produced cyanide, varied considerably between the replicates (13–83%). Means of eight replicates per treatment did not differ significantly (Table 1).

Pseudomonas fluorescens isolate WCS374 and *P. putida* isolate WCS358 did not produce cyanide on KB or on KB- or TSA-supplemented with glycine, either in the original or in the modified Castric media. *Pseudomonas* sp. isolate WCS361 did produce cyanide on these media. On King's medium B, growth of the five HCN-producing *Pseudomonas* spp isolates was strongly inhibited within the fluorescent zones around colonies of WCS358 or WCS374.

In short potato-rotation soil cropped to potato, cyanide could not be detected by the techniques described.

Cyanide sensitivity of cytochrome oxidase respiration of potato roots

Addition of KCN (final concentration 5 μM) to a mineral salts solution containing 25 mM SHAM reduced the O_2 consumption rate of intact excised potato roots in this solution to 60%, compared with controls to which no KCN was added (Table 2). Concentrations of KCN lower than 5 μM also caused a reduction of the O_2 consumption rate, but in these cases the results were more variable.

Table 1. Percentage cyanide-producing Pseudomonads isolated from potato and wheat rhizosphere in short (1:1) and long (1:6) potato rotation soil

Origin	Pseudomonads producing cyanide (%)[1] 1:1 rotation	1:6 rotation
Potato rhizosphere	55[a]	59[a]
Wheat rhizosphere	37[a]	48[a]

[1]Isolates growing on KB^+ producing cyanide on KB + glycine, as percentage of total numbers of isolates growing on KB^+ (pseudomonads); 960 isolates were screened for cyanide production. Figures followed by the same character do not differ significantly [$\alpha = 0.05$].

Table 2. The effect of cyanide on cytochrome oxidase respiration of potato roots

μM KCN added[1]	O_2 consumption rate of roots in 25 mM SHAM[2] (%)
0 (control)	100
1	91[3] (SD = 5.8)
2.5	76[3] (SD = 8.5)
5	60[4] (SD = 1.4)

[1]KCN was added in 0.1 M NaOH solution.
[2]pH of mineral salts solution containing 25 mM salicylhydroxamic acid was 7.40, $T = 20°C$.
[3]Average of two replicates. (SD: standard deviation).
[4]Average of four replicates.

Effect of Fe^{3+} concentration on HCN production by Pseudomonas *sp. isolate WCS361*

Amounts of biomass of *Pseudomonas* sp. isolate WCS361 and of cyanide produced after 5 days incubation were at highest at the highest $FeCl_3$ concentration (10^{-4} mol $FeCl_3$ added l^{-1}) (Fig. 2A), being 0.8 g dry weight cells l^{-1} culture and 460 μmol HCN l^{-1} culture, respectively. Biomass and cyanide production both decreased with decreasing $FeCl_3$ concentration. Cyanide production per unit of biomass also decreased with decreasing $FeCl_3$ concentration, except for the lowest concentration (Fig. 2B). Addition of 0.3 mmol NaCl l^{-1} medium did not change cyanide and biomass production compared to the treatment without addition of NaCl.

DISCUSSION

Only a small proportion (±5%) of the total number of bacteria from wheat and potato rhizosphere, isolated on TSA, were able to produce cyanide. They grew well on KB^+, a medium selective for *Pseudomonas* spp, which suggests that they are pseudomonads. Of the rhizosphere bacteria isolated on KB^+, more than 50% were cyanide producing. Apparently, the ability to produce cyanide is most common among rhizosphere pseudomonads.

Cyanide could not be detected in the rhizosphere of potato with the methods used. However, the assimilation and detoxification of cyanide by soil microorganisms (Castric, 1981) makes the chances of demonstrating microbial cyanide in the rhizosphere by our technique very small. This does not necessarily detract from any supposed effect of microbial cyanide on root cell metabolism. Much of the cyanide produced by bacteria in direct contact with root cells, will not be available for microbial degradation, as it most likely diffuses directly into the root cells. Cyanide may also be produced by pseudomonads in the endorhizosphere, where they can be abundant (Jacobs *et al.*, 1985).

Respiration by the cytochrome pathway in intact potato roots *in vitro* appears to be very sensitive to cyanide. When the alternative electron transport pathway was inhibited by SHAM (Lambers, 1980), the remaining oxygen consumption rate was reduced to 60% by 5 μM KCN. In SHAM solution, root O_2 consumption is partly due to the activity of cyanide-sensitive cytochrome oxidase and partly to other oxidases which are cyanide-insensitive (Theologis and Laties, 1978). This implies that when O_2 consumption of potato roots in SHAM solution is reduced to 60% by the addition of KCN, more than 40% of the cytochrome pathway respiration is inhibited.

The supposition, that yield reductions may partly originate from impaired nutrient uptake caused by a depressed root cell energy metabolism, corresponds with the observations in field experiments at the "De Schreef" experimental farm. In these experiments P and K concentrations in potato plants from short potato-rotations were significantly lower than those

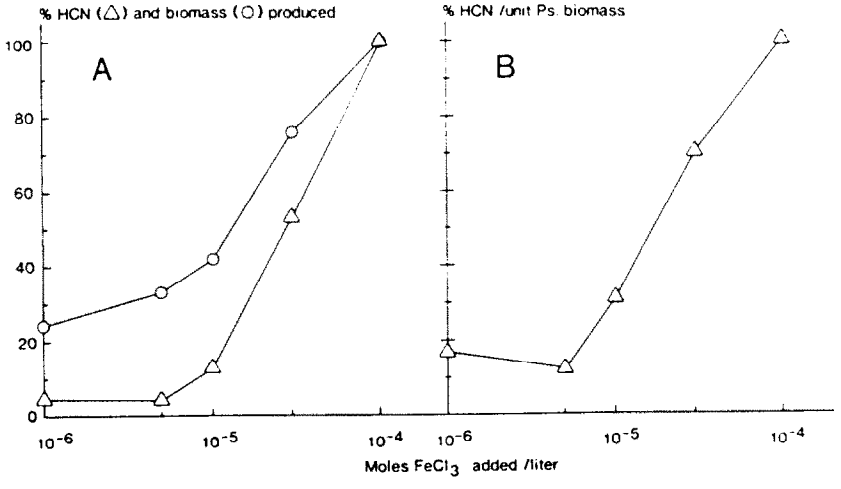

Fig. 2. (A) Cyanide and biomass produced by *Pseudomonas* sp. isolate WCS361 at different Fe^{3+} concentrations during a 120 h incubation, expressed as percentages of cyanide and biomass, respectively, produced at the highest Fe^{3+} concentation. Cyanide produced at 10^{-6}, 5.10^{-6}, 10^{-5}, 3.10^{-5} and 10^{-4} mol $FeCl_3$ added l^{-1} medium was: 4% (4%), 4% (3%), 13% (4%), 53% (12%) and 100% (20%) respectively. Biomass produced was: 24% (12%), 33% (11%), 42 (17%), 76 (12%) and 100% (23%) respectively. The figures in parenthesis are standard deviations. (B) Cyanide produced per unit of *Pseudomonas* sp. isolate WCS361 biomass at different Fe^{3+} concentrations during 120 h of incubation, expressed as percentage of cyanide production at the highest Fe^{3+} concentration.

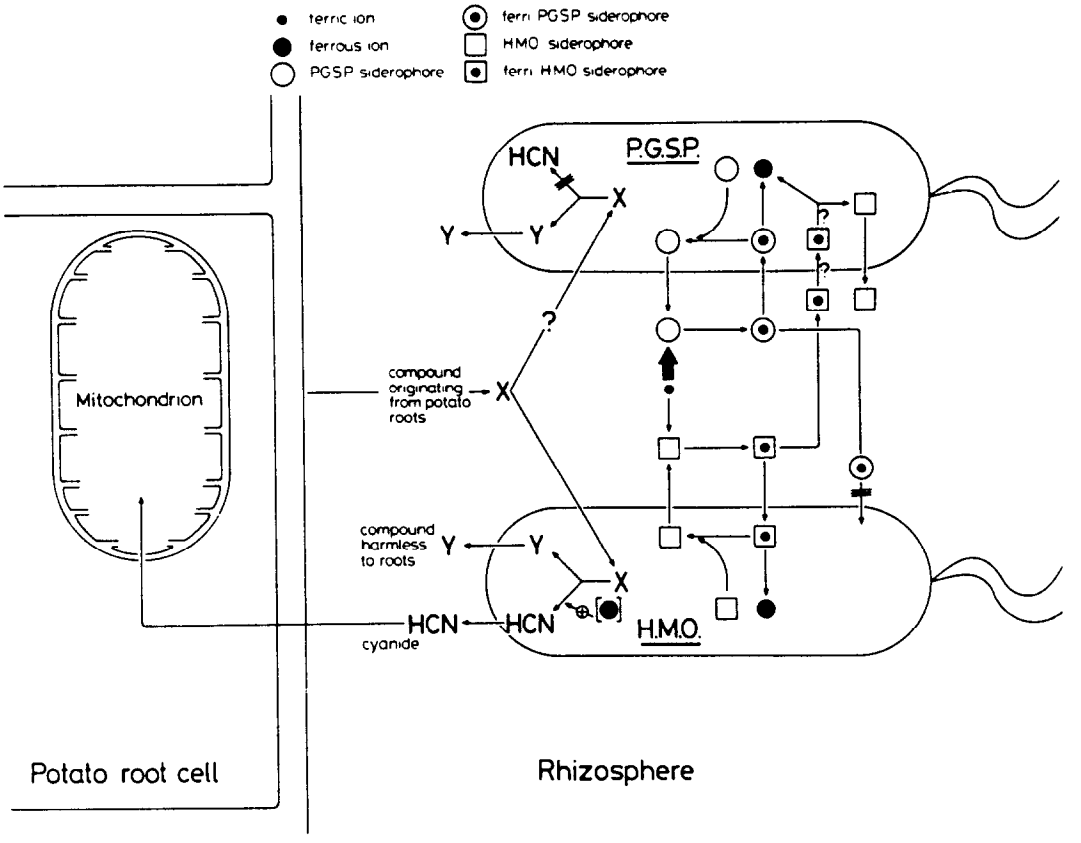

Fig. 3. Hypothetical mechanisms for (a) inhibition of potato root functioning by cyanide-producing harmful micro-organisms (HMO) in short potato-rotation soil, and (b) for mitigation of this inhibition by plant growth-stimulating pseudomonads (PGSP) which operate through siderophore-mediated competition for iron, inhibiting cyanide production. [●]: concentration of ferrous ion; ⊕: stimulation of the process by the factor involved.

in potato plants from long potato-rotations (J. Vos and K. Groenwold, personal communication). The mobility of P and K in soil is low (Ayres, 1984). Reduced root development may therefore reduce P and K concentrations in potato plants. At "De Schreef", however, this does not seem to be the case, since no differences in total root length could be demonstrated between different potato rotations (J. Vos and K. Groenwold, personal communication).

In contrast to the situation in the field, the root development of potato stem cuttings in the bioassay was reduced in short potato-rotation soil, compared to that in the long rotation soil (P. A. H. M. Bakker *et al.*, 1987). In the bioassay, reduced nutrient uptake is likely to be reflected almost immediately in decreased root development because nutrient reserves in the newly-rooted stem cuttings must be very low. In field experiments, however, nutrient reserves in the seeded tuber may prevent such an effect. In the bioassay, therefore, the reduced root development is rather a *consequence* of reduced nutrient uptake due to impaired energy metabolism, than the *cause* of it.

Potato yield increases induced by fluorescent pseudomonads have frequently been ascribed to siderophore-mediated competition for Fe^{3+} with unknown harmful rhizosphere microorganisms (Kloepper *et al.*, 1980; Geels and Schippers, 1983c; Schippers *et al.*, 1985, 1986). Evidence has now been obtained that siderophore production is a prerequisite for root growth-stimulation of potato stem cuttings by isolate WCS358 in short potato-rotation soil (P. A. H. M. Bakker *et al.*, 1987). It is therefore very likely that this growth stimulation is due to decreased iron availability for rhizosphere microorganisms harmful to roots. In this respect it is noteworthy that cyanide production by *Pseudomonas* sp. isolate WCS361 is dependent on Fe^{3+} availability (Fig. 2). This observation agrees with that of Castric (1975) for *Pseudomonas aeruginosa*. Moreover, growth of each of five cyanide-producing rhizosphere *Pseudomonas* spp isolates could be inhibited by *Pseudomonas* spp isolates WCS358 and WCS374 on KB. Neither of these two isolates (both of which stimulate plant growth) produced cyanide in *in vitro* experiments. The cyanide-producing isolate WCS361, although a strong siderophore-mediated competitor for Fe^{3+} on KB, could not counteract the yield reductions in short potato-rotation soil significantly (Geels and Schippers, 1983a, c). These observations, and (1) the high percentage of rhizosphere pseudomonads with the potential to produce cyanide (Table 1) and, (2) the high cyanide sensitivity of cytochrome oxidase in intact potato roots to cyanide (Table 2), contribute to the hypothesis that cyanide-producing micro-

organisms are involved in the reduction of plant development and of tuber yield of potato crops in our short potato-rotation soils. That no differences were found between numbers of HCN-producing pseudomonads isolated from roots from long and short potato-rotation soils does not detract from this argument. What matters is the rate of cyanide-production by these bacteria. The rate of HCN-production by pseudomonads depends on several environmental factors (Knowles, 1976; Castric, 1975). One or more of these factors may be different in the different rotation soils, leading to different rates of HCN-production, even though numbers of HCN-producing pseudomonads may be equal. Other possibilities are: more siderophore-producing pseudomonads antagonistic to HCN-producing pseudomonads occur in long rotation soil; or relatively more "strong" HCN-producers develop in short potato-rotation soil.

Competition between organisms will increase with increasing overlap between their ecological niches (Krebs, 1972). As both the cyanide-producing isolates and the growth-promoting isolates WCS358 and WCS374 are fluorescent pseudomonads isolated from the rhizosphere of potato, their ecological niches are likely to overlap, as will the actual locations where they are active. Considering this and the dependence of cyanide production by pseudomonads on iron availability, potato growth stimulation by *Pseudomonas* spp isolates WCS358 and WCS374 in short potato rotations may be due, at least partly, to siderophore-mediated suppression of microbial cyanide production. This hypothesis is presented schematically in Fig. 3.

Our hypothesis, that microbially-produced HCN reduces potato yield, implies the accumulation in short potato rotation soils of as yet unknown factors which enhance microbial HCN-production. It is possible that the number of HCN-producing microorganisms increase in short potato-rotation soils during the growing season. This question cannot be settled from the present work and must be the subject of further research. Increasing production of cyanide does not necessarily originate from increasing *numbers* of cyanide producers. It is more likely due to an alteration in the secondary metabolism of potential HCN-producing pseudomonads, leading from no or little HCN-production to (increased) production of HCN. Such a change could be caused by accumulation of as-yet-unknown substances in short potato-rotation soils. Such substances could, for example, act as precursors of cyanide, or increase the availability of Fe^{3+} and thereby the HCN-production. This is the subject of further research.

In conclusion, we propose new explanations for the way in which certain saprophytic rhizosphere pseudomonads can restrict plant growth and for how this damage can be mitigated by siderophore-mediated competition for Fe^{3+}.

Acknowledgements—We thank Miss M. A. Verhaar, Mr J. L. Broos and Mr P. M. Lammers for experimental assistance, Mrs M. A. Williamson for correcting the English text, Mr P. A. H. M. Bakker for critical comments and suggestions and Mr J. van den Heuvel for critically reading the manuscript.

REFERENCES

Ayres P. G. (1984) Effects of infection on root growth and function; consequences for plant nutrient and water relations. In *Plant Diseases—Infection, Damage and Loss* (R. K. S. Wood and G. J. Jellis, Eds), pp. 105–118. Blackwell, Oxford.

Bakker P. A. H. M., Bakker A. W., Marugg J. D., Weisbeek P. J. and Schippers B. (1987) Bioassay for studying the role of siderophores in potato growth stimulation by *Pseudomonas* spp in short potato rotations. *Soil Biology & Biochemistry* **19**, 443–449.

Castric P. A. (1975) Hydrogen cyanide, a secondary metabolite of *Pseudomonas aeruginosa*. *Canadian Journal of Microbiology* **21**, 613–618.

Castric P. A. (1981) The metabolism of hydrogen cyanide by bacteria. In *Cyanide in Biology* (B. Vennesland, E. E. Conn, C. J. Knowles, J. Westley and F. F. Wissing, Eds), pp. 233–261. Academic Press, New York.

Curl E. A. and Truelove B. (1985) *The Rhizosphere*. Springer-Verlag, Berlin.

Geels F. P. and Schippers B. (1983a) Reduction of yield depressions in high frequency potato cropping by fluorescent *Pseudomonas* spp. *Les Colloques de l'INRA* **18**, 231–238.

Geels F. P. and Schippers B. (1983b) Selection of antagonistic fluorescent *Pseudomonas* spp. and their root colonization and persistence following treatment of seed potatoes. *Phytopathologische Zeitschrift* **108**, 193–206.

Geels F. P. and Schippers B. (1983c) Reduction of yield depressions in high frequency potato cropping soil after seed tuber treatments with antagonistic fluorescent *Pseudomonas* spp. *Phytopathologische Zeitschrift* **108**, 207–214.

Hoekstra O. (1981) 15 jaar "De Schreef". Resultaten van 15 jaar vruchtwisselingsonderzoek op het bouwplannenproefveld "De Schreef". *Publikatie PAGV* **11**, 1–93. (Lelystad Alkmaar, Nederland).

Jacobs M. J., Bugbee W. M. and Gabrielson D. A. (1985) Enumeration, location and characterization of endophytic bacteria within sugar beet roots. *Canadian Journal of Botany* **63**, 1262–1265.

King E. O., Ward M. K. and Raney D. E. (1954) Two simple media for the demonstration of pyocyanin and fluorescin. *Journal of Laboratory and Clinical Medicine* **44**, 301–307.

Kloepper J. W., Leong J., Teintze M. and Schroth M. N. (1980) Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature, London* **286**, 885–886.

Knowles C. J. (1976) Microorganisms and cyanide. *Bacteriological Reviews* **40**, 652–680.

Krebs C. J. (1972) *Ecology*, 1st ed, Harper & Row, New York.

Lambers H. (1980) The physiological significance of cyanide-resistant respiration in higher plants. *Plant, Cell and Environment* **3**, 293–302.

Lambers H., Blacquière T. and Stuiver C. E. E. (1981) Interactions between osmoregulation and the alternative respiratory pathway in *Plantago coronopus* as affected by salinity. *Physiologia Plantarum* **51**, 63–68.

Lamers J. G. (1981) Continuteelt en nauwe rotaties van aardappelen en suikerbieten. *Publikatie PAGV* **12**, 1–65.

Meyer J. M. and Abdallah M. A. (1978) The fluorescent pigment of *Pseudomonas fluorescens*: biosynthesis, purification and physicochemical properties. *Journal of General Microbiology* **107**, 319–328.

Nagashima S. and Ozawa T. (1981) Spectrophotometric determination of cyanide with isonicotinic acid and barbituric acid. *International Journal of Environmental Analytical Chemistry* **10**, 99–106.

Rovira A. D. and Davey C. B. (1974) Biology of the rhizosphere. In *The Plant Root and its Environment* (E. W.

Carson, Ed.), pp. 153–204. University Press of Virginia, Charlottesville.

Schippers B., Geels F. P., Hoekstra O., Lamers J. G., Maenhout C. A. A. A. and Scholte K. (1985) Yield depressions in narrow rotations caused by unknown microbial factors and their suppression by selected pseudomonads. In *Ecology and Management of Soil-Borne Plant Pathogens* (C. A. Parker, A. D. Rovira, K. J. Moore, P. T. W. Wong and J. F. Kollmorgen, Eds), pp. 127–130. The American Phytopathological Society, St Paul, Minnesota.

Schippers B., Bakker A. W., Bakker P. A. H. M., Weisbeek P. J. and Lugtenberg B. (1986) Plant growth-inhibiting and stimulating rhizosphere micro-organisms. In *Microbial Communities in Soil* (V. Jensen, A. Kjøller and L. H. Sørensen, Eds), pp. 35–48. FEMS/Elsevier, Amsterdam.

Scholte K., Veenbaas-Rijks J. W. and Labruyère R. E. (1985) Potato growing in short rotations and the effect of *Streptomyces* spp., *Colletotrichum coccodes*, *Fusarium tabacinum* and *Verticillium dahliae* on plant growth and tuber yield. *Potato Research* **28**, 331–348.

Theologis A. and Laties J. J. (1978) Relative contribution of cytochrome-mediated and cyanide resistant electron transport in fresh and aged potato slices. *Plant Physiology* **62**, 232–237.