Biological Control of Fungal Strawberry Diseases by *Serratia plymuthica* HRO-C48

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ABSTRACT

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To develop a biological control product for commercial strawberry production, the chitinolytic rhizobacterium *Serratia plymuthica* strain HRO-C48 was evaluated for plant growth promotion of strawberries and biological control of the fungal pathogens *Verticillium dahliae* and *Phytophthora cactorum*. In phytochamber experiments, treatment with *S. plymuthica* HRO-C48 resulted in a statistically significant enhancement of plant growth dependent on the concentration of the bacterium that was applied. In greenhouse trials, bacterial treatment reduced the percentage of Verticillium wilt (18.5%) and Phytophthora root rot (33.4%). In three consecutive vegetation periods, field trials were carried out in soil naturally infested by both soilborne pathogens on commercial strawberry farms located in various regions of Germany. Dipping plants in a suspension of *S. plymuthica* prior to planting reduced Verticillium wilt compared with the nontreated control by 0 to 37.7%, with an average of 24.2%, whereas the increase of yield ranged from 156 to 394%, with an average of 296%. Bacterial treatment reduced Phytophthora root rot by 1.3 to 17.9%, with an average of 9.6%, and increased strawberry yield by 60% compared with the nontreated control. Under field conditions, strain HRO-C48 survived at approximately $\log_{10}$ 3 to 7 CFU/g of root in the strawberry rhizosphere at 14 months after root application. Although results of the field trials were influenced by pathogen inoculum density, cropping history of the field site, and weather conditions, *S. plymuthica* HRO-C48 successfully controlled wilt and root rot of strawberry.

Strawberry (*Fragaria* × *ananassa* Duch.) is an important high-value culturable crop. In recent years, an increase in strawberry production has been recorded worldwide (FAO, Statistical Databases). Verticillium wilt caused by *Verticillium dahliae* Kleb. and root rot caused by *Phytophthora cactorum* (Lebert & Cohn) J. Schröt. are important diseases responsible for dramatic yield losses in commercial strawberry production (24). Microsclerotia of *V. dahliae* that develop in the senescing tissues of the dead plant may persist in soil for several years in the absence of a susceptible host; therefore, chemical control is nearly impossible (24). In the coming years, the loss of methyl bromide as a control measure for Verticillium wilt will have a great impact on the accumulation of microsclerotia in soil (13). Efficacious control methods are urgently needed for commercial strawberry production.

An environmentally friendly alternative to protect roots against fungal pathogens is rhizobacteria-mediated biological control (7,12,34). Numerous studies have demonstrated the ability of several rhizobacteria to suppress diseases caused by fungal plant pathogens (10,29,34,35). Isolates of the gram-negative bacterial genus *Serratia* are frequently found associated with plant roots and possess antifungal properties (15,26,27). Therefore, they have potential for the biological control of plant pathogens. For example, *Serratia liquefaciens* from carnation rhizosphere is used to protect root cuttings (31), and *S. marcescens* B2 is able to control cyclamen soilborne diseases (32). *S. plymuthica* has been reported to exhibit antifungal effects against *Fusarium culmorum, Pythium* spp., and other fungal pathogens (1,4,18,22).

S. plymuthica strain HRO-C48 was isolated from the rhizosphere of oilseed rape (18) and selected as a biocontrol agent according to the following criteria: (i) high chitinolytic activity responsible for activity against fungal pathogens (e.g., *V. dahliae* and *Phytophthora cactorum*) in vitro (6,11,18); (ii) production of the plant growth hormone indole-3-acetic acid (18); (iii) relative harmlessness to human health and the environment (3); and (iv) low level of antibiotic resistance (5). The use of the antifungal properties of *Serratia* spp. for biocontrol of plant pathogens has been widely reported in scientific reports (4,18,19,21,25) and also in patents (8,20). It is surprising that no biocontrol product based on *S. plymuthica* is currently available on the market (see United States Department of Agriculture web site). Members of the species *S. plymuthica* were evaluated in only a few pathosystems (e.g., cucumber–*Pythium ultimum;* 25), and biocontrol results obtained under field conditions have never been reported.

The objective of our study was to evaluate *S. plymuthica* strain HRO-C48 for biocontrol of Verticillium wilt and Phytophthora root rot in strawberry. During three consecutive years, the efficacy of this biocontrol strain in phytochamber, greenhouse, and field trials on three different locations naturally infested by *Verticillium* or *Phytophthora* spp. was analyzed. The field trials were integrated into commercial strawberry production.

MATERIALS AND METHODS

Identification and culturing of bacteria and fungi. *S. plymuthica* was isolated from the rhizosphere of oilseed rape (18). A spontaneous mutant isolate of *S. plymuthica* resistant to rifampicin HRO-C48*Rif*^{r} (100 µg/ml; Fluka, Buchs, Switzerland) was used in all experiments. No differences in colony morphology, antifungal properties, and growth rate were found between the mutant and wild type. The strains of *S. plymuthica* were stored at –70°C in nutrient broth (nutrient broth 2 = 6.75 g of peptone, 5 g of NaCl, 1.5 g of yeast extract, 1.75 g of hydrolyzed protein in 1 liter of distilled water, 30°C, pH 7.2; Sifin, Berlin, Germany) containing 15% glycerol. Identification using the API system (BioMérieux, Marcy l'Etoile, France) resulted in an identification rate of 57.5% (profile no. 1006323). With the Biolog system (BIOLOG Inc., Haward, CA), the strain was identified with an identification rate of 86.3%. Additionally, the 16S rRNA was sequenced and aligned with the reference 16S rRNA gene sequence using BLAST algorithm according to Altschul et al. (2). In comparison with the strain *S. plymuthica* PSM 4540 (EMBL Accession number AJ 2334331), strain HRO-C48 was determined to be *S. plymuthica* with 98%

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identity. The isolate was deposited in the German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung für Mikroorganismen und Zellkulturen, Braunschweig, Germany) as DSMZ 12502. For greenhouse trials, the fungal strain *Phytophthora cactorum* PF-8 was obtained from the Federal Biological Research Center for Agriculture and Forestry (BBA, Darmstadt, Germany). The fungus was routinely grown on V8 juice agar at 16°C and stored in V8 juice broth with 15% glycerol at –70°C.

Phytochamber assay for effect of *S. plymuthica* on plant growth. Seeds of strawberry cv. Rügen Selecta (Erfurter Samenzucht, Erfurt, Germany) were pregerminated in moist chambers at 30°C for 6 days. Three standard 24-well microplates (Roth, Karlsruhe, Germany) were filled with 1 ml of water agar containing 20 g of agar (Difco, Detroit, MI) made up to 1 liter with distilled water (pH 6.8). One pregerminated seed followed by 10 µl of bacterial suspension (nutrient broth 2, grown for 18 h) was added to each well. *S. plymuthica* was evaluated at 10^3, 10^5, 10^7, and 10^9 CFU/ml and compared with a control of 10 µl of distilled water. Five weeks after incubation (16 h of artificial light, 22°C) in a chamber (Percival Scientific, Boone, IA), first leaves and radial roots were counted to determine effects of bacterial treatment on plant growth. The strain was tested in six replicates at each concentration, and the experiment was repeated three times.

Biological control and growth promotion by *S. plymuthica* in the greenhouse. Soil naturally infested by *V. dahliae* (from the location in Stuthof, field trial I) or artificially infested by *P. cactorum* were used to evaluate biological control potential of *S. plymuthica*. Sterilized soil (sandy loam, pH 6.4) was infested with the pathogenic fungus *P. cactorum* PF-8 according to Hessenmüller and Zeller (17). Mycel plugs of *P. cactorum* PF-8 were inoculated in a 4-liter mixture of wheat bran:vermiculite (1:1) containing 240 g of soybean flour and 668 ml of distilled water. This mixture was incubated for 28 days at 22°C. Polypropylene boxes (0.7-liter) were filled with soil and *Phytophthora* inoculum (4%) and planted with strawberry frigo cv. Elsanta plants (Janssen, Kalkar, Germany). Prior to planting, the roots were dipped in a suspension of *S. plymuthica* (2×10^9 CFU/ml) for 15 min. The nontreated control plants were dipped in tap water and planted in infested soil. A negative control using pathogen-free soil was included in each test. Ten replicates of each treatment were performed in a completely randomized block design. All treatment combinations were repeated three times. The experiments were conducted under greenhouse conditions (18 h of light, sodium lamps, 100 mE/m^2/s, 25 ± 1°C) over a 10-week period. After inoculation, disease incidence based on a 0-to-2 scale, with 0 = no disease, 1 = infected plant showing wilting symptoms, and 2 = dead plants, was recorded and the number of buds and blossoms were counted. To evaluate the influence of the treatment on yield, the weights of fruit were measured. At the end of the 10-week trial, plant roots with adhering soil taken from five plants per treatment were aseptically sampled to sterile Stomacher bags. Each sample (5 g) was extracted in a Stomacher laboratory blender (BagMixer, Interscience, St. Nom, France) with sterile NaCl solution (8.5 g per liter). Solutions were serially diluted and plated on nutrient agar 2 containing rifampicin (Fluka) at 100 µg/ml. CFU were determined after a 5-day incubation at 20°C. The total culturable bacterial populations in the rhizosphere were determined using the same procedure on nutrient agar.

Biological control of Verticillium wilt and Phytophora root rot in field trials. Field trials I (1997, location Stuthof) and II (1998, location Goorstorf) were conducted in Mecklenburg-Western-Pomerania, Germany, in areas naturally infested by *V. dahliae.* Soil population density of *V. dahliae* in Stuthof and Goorstorf was 40 and 21 microsclerotia/g of soil, respectively, determined by Termorshuizen's soil dilution method (33). In 1999, field trial III was carried out in Bühl, Baden-Württemberg, in a field naturally infested by *P. cactorum* at 1,000 CFU/g of soil (17). Soil parameters at all locations were analyzed by the Institute for Agricultural Analysis and Research (LUFA, Rostock or Bonn, Germany). In Stuthof (trial I), the soil texture was loamy sand, pH 5.5, 1.7% organic matter, with the following nutrients in milligrams per 100 g of soil: P_2O_5, 12; K_2O, 15; and Mg, 6. In Goorstorf (trial II), the soil texture was sand, pH 5.7, 1.3% organic matter, with the following nutrients in milligrams per 100 g of soil: P_2O_5, 16; K_2O, 8; and Mg, 11. In Bühl (trial III), the soil texture was sandy loam, pH 5.1, with the following nutrients in milligrams per 100 g of soil: P_2O_5, 24; K_2O, 33; and Mg, 5. In trials I and II, approximately 1,200 strawberry cv. Elsanta plants were grown in a completely randomized block design with six replicates. The strawberries were planted as frigo plants in May, harvested as runner plants in autumn, and stored at –2°C during the winter. In trial III, approximately 100 strawberry cv. Elsanta plants per treatment were grown in a completely randomized block design with four replicates. The roots of strawberry plants were dipped in a bacterial suspension (4×10^9 CFU/ml) immediately prior to planting. Controls were dipped in tap water. In July of the same year, the strawberry fruits were harvested and weighed to determine yield and the percentage of wilted and dead plants was monitored. In field trial III, the strawberries were planted as runner plants in August and treated with *S. plymuthica* as described above. Disease incidence (based on a 0-to-2 scale, with 0 = no disease, 1 = infected plant showing wilting symptoms, and 2 = dead plants) was recorded in September and October of 1999. At four different sampling times (young plants, flowering plants, fruiting plants, and flowering plants in the second year after planting), plant roots with adhering soil taken from five plants from one treatment were aseptically sampled to sterile Stomacher bags and treated as one sample. Five replicates for each treatment were investigated. Each sample (5 g) was extracted in a Stomacher laboratory blender (BagMixer) with sterile NaCl solution (8.5 g per liter). Solutions were serially diluted and plated on nutrient agar 2 containing rifampicin at 100 ppm (Fluka). CFU were determined after a 5-day incubation at 20°C. The total culturable bacterial populations in the rhizosphere was determined using the same procedure on nutrient agar. Weather data were obtained from the State Agency of Environment, Nature Conversation, and Geology, Mecklenburg-Western-Pomerania (LUNG, Güstrow, Germany) and the Deutscher Wetterdienst (Offenbach, Germany).

Statistical analysis. Data on the percentages of disease incidence and yield were analyzed for significance using U-Test "Mann-Whitney" ($P \leq 0.05$) by Statistical Product and Service Solutions for Windows, Rel. 9.0.1. (SSPS Inc., Chicago). Root colonization data were $\log_{10}$ transformed before statistical analysis. A polynomic regression analysis was used to compare the treatments at each *S. plymuthica* concentration in the phytochamber assay.

RESULTS

Plant growth promotion in phytochamber. The effect of *S. plymuthica* on plant growth was dependent on the bacterial population applied on germ buds (Fig. 1). Germ buds treated with a bacterial population at a concentration of 10^3 CFU/ml had more first leaves ($P = 0.000$) and radial roots ($P = 0.009$) compared with the nontreated control. The plant growth promotion effect was also shown at higher bacterial concentrations (10^5 CFU/ml: leaves [$P = 0.000$] and roots [$P = 0.002$]; 10^7 CFU/ml: leaves [$P = 0.006$] and roots [$P = 0.095$]). At bacterial populations >10^7 CFU/ml, the percentage of leaves and radial roots was not different from the nontreated control. The dose effect for the increase of leaves was significant. Significant differences in the increase of leaves were found between the *S. plymuthica* concentrations applied.

Effects of *S. plymuthica* HRO-C48 on disease incidence and strawberry growth and yield under greenhouse conditions. In soil naturally infested by *V. dahliae* (greenhouse trial I) and artificially inoculated by *P. cactorum* (greenhouse trial II), treating plants with *S. plymuthica* re-

duced the number of wilted and necrotized plants (Table 1). Ten weeks after treatment, the average reduction of Verticillium wilt was 8.2% and the reduction of Phytophthora root rot was 3.6% compared with the nontreated control. Plants treated with *S. plymuthica* and planted in soils infested by *V. dahliae* or *P. cactorum* had 33.2 and 16.2% more buds and blossoms, respectively, than the nontreated control. In the nontreated controls of both trials, the initiation of flowering was delayed. In both trials, yield was increased by treatment with *S. plymuthica* (Table 1). Yield was increased by 18.5% in soil naturally infested by *Verticillium* spp. and, in soils infested with *P. cactorum,* yield was increased by 33.4%.

After 10 weeks, the population of *S. plymuthica* HRO-C48 and the total bacterial populations in the strawberry rhizosphere were determined. *S. plymuthica* strain HRO-C48 colonized the treated roots of strawberry at population densities between 5.4 and 6.6 $\log_{10}$ CFU/g of root. *S. plymuthica* was not detected in the nontreated control rhizospheres. The total bacterial populations in the rhizosphere ranged from 6.2 to 7.1 $\log_{10}$ CFU/g of root and were not different among treatments.

Effect of *S. plymuthica* HRO-C48 on disease incidence and strawberry growth and yield in field trials. Three field trials at different locations were carried out to evaluate the ability of *S. plymuthica* HRO-C48 to suppress the pathogens under natural conditions. In field trial I (1997, Stuthof), wilting symptoms of *V. dahliae* were reduced (0 to 37.7%) in plots treated with strain *S. plymuthica* (92 diseased plants to 235 healthy plants) compared with nontreated control plots (203 diseased plants to 190 healthy plants) (Fig. 2). In field trial II (1998, Goorstorf), symptoms of *V. dahliae* did not develop. Differences in disease incidence between the control and *S. plymuthica* HRO-C48-treated plants were not detected. In field trial III, the reduction of Phytophthora root rot by the treatment with *S. plymuthica* compared with the nontreated control ranged from 1.3 to 17.9%, with an average of 9.6% (Fig. 2). In field trial I (1997), the yield of strawberries in plants treated with *S. plymuthica* (32.5 kg per 100 plants) was 296% greater than in the nontreated control (8.2 kg per 100 plants). In field trial II (1998, Goorstorf), yield was not different between the control and plants treated with *S. plymuthica*. In field trial III, plants treated with *S. plymuthica* exhibited greater growth 1 month after planting in September and in October.

S. plymuthica HRO-C48 was reisolated throughout the 14-month period (Fig. 3) and identified using the API and BIOLOG systems. In field trial I, *S. plymuthica* was recovered at the second (flowering plants) and third (fruiting plants) sampling periods. For both field trials I (1997, Stuthof) and II (1998, Goorstorf), *S. plymuthica* HRO-C48*Rif*^{r} decreased each period. Fourteen months after planting, *S. plymuthica* was detected at significantly lower levels ($\log_{10}$ 2.9 CFU/g of root in Stuthof in 1998 [field trial I]). In 1999, 2 years after inoculation, the strain was not recovered from the soil in Goorstorf (field trial II). In the field trials, the total bacterial populations were investigated to monitor the influence of the introduced strain on the bacterial community. Total bacterial populations recovered from the rhizospheres treated with *S. plymuthica* were similar to the nontreated control.

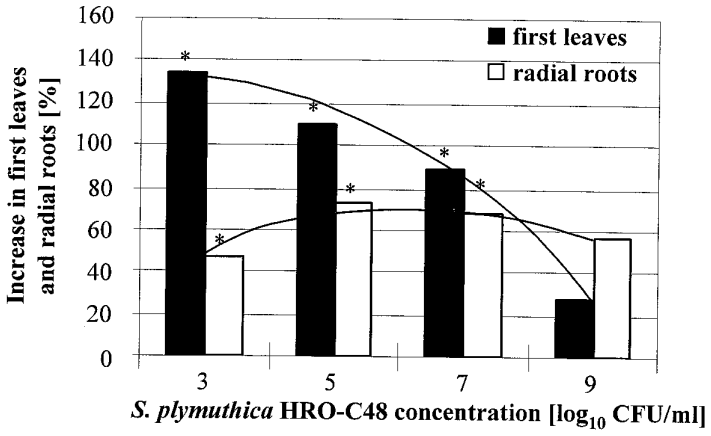

Fig. 1. Influence of different concentrations of *Serratia plymuthica* strain HRO-C48; 3 weeks after treatment on the development of strawberry first leaves and radial roots compared with a nontreated control in a phytochamber assay. A suspension of *S. plymuthica* (10 µl) at concentrations of 10^3, 10^5, 10^7, and 10^9 CFU/ml was applied on each of the 72 seeds per treatment. Significant differences ($P \leq 0.05$) were determined by Mann-Whitney and are indicated by asterisks.

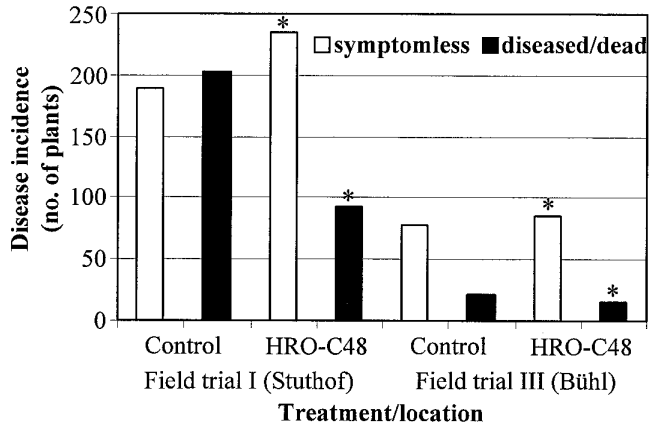

Fig. 2. Numbers of symptomless and diseased or dead plants treated with *Serratia plymuthica* strain HRO-C48 compared with a nontreated control in randomized field trials (I = 1997, Stuthof; II = 1998, Goorstorf; and III = 1999, Bühl). *S. plymuthica* strain HRO-C48 was applied by dipping the roots in a suspension (4×10^9 CFU/ml) immediately prior to planting. Disease symptoms were monitored in September, 1 month after harvest (fields I and II) or 1 month after planting (field trial III). Significant differences ($P \leq 0.05$) were determined by Mann-Whitney and are indicated by asterisks.

Table 1. Effect of *Serratia plymuthica* strain HRO-C48 on disease incidence and strawberry growth (numbers of buds and blossoms) and yield in greenhouse trials using soil naturally infested by *Verticillium dahliae* or artificially infested by *Phytophthora cactorum*[a]

Trial no., treatment	Disease incidence (SE)	No. of buds and blossoms (SE)	Yield (SE)
I			
Negative control	0 (±0.044)	3.1 (±2.2)	11.6 (±0.5)
Nontreated control	0.926 (±0.09)	0.76 (±1.1)	5.1 (±1.1)
HRO-C48-treatment	0.76 (±0.082)	1.79 (±1.9)	7.2 (±1.8)
II			
Negative control	0 (±0.044)	3.1 (±2.2)	11.6 (±0.5)
Nontreated control	0.562 (±0.08)	0.67 (±1.2)	1.7 (±1.5)
HRO-C48-treatment	0.49 (±0.084)	3.03 (±2.1)	5.6 (±0.7)

[a] Disease incidence, number of buds and blossoms, and yield (g) ± standard error (SE).

Climatic aspects. Weather conditions during field trials I (1997), II (1998), and III (1999) were different (Fig. 4). The vegetation period in 1997 started with a cold and rainy spring (May: temperature = 10.8°C, rainfall = 2.4 mm) during planting. This period was followed by a warm, dry summer with a maximum temperature of 21°C in August and low rainfall (1.9 mm) during the flowering period and harvest. In contrast, the spring of 1998 was not as cold as the spring of 1997 (May = 12.9°C) and the total rainfall level was lower (1.4 mm). In 1998, during the flowering period and harvest, the temperature between June and October was generally lower (13.9°C on average) compared with 1997 (15.4°C). The amount of rainfall from June to October was higher than the year before. After harvest at the end of October, the conditions in both years (1997 and 1998) were similar, with an average temperature of 8.7°C day^{-1}. The level of rainfall per month ranged from 1.4 to 1.9 mm. The third trial, located in Bühl (Baden-Württemberg), had higher average temperatures and rainfall per month than the other trails.

DISCUSSION

Potential use of root-colonizing bacteria as replacements or supplements for chemical fungicides have been addressed in many reports (10,29,34,35). In the present study, the potential of a strain of *S. plymuthica* isolated from the rhizosphere of oilseed rape for controlling Verticillium wilt and Phytophthora root rot of strawberries in greenhouse and field trials was evaluated over a 3-year period.

A requirement for an efficient biological control agent is the ability to survive and to become established in the rhizosphere (23).

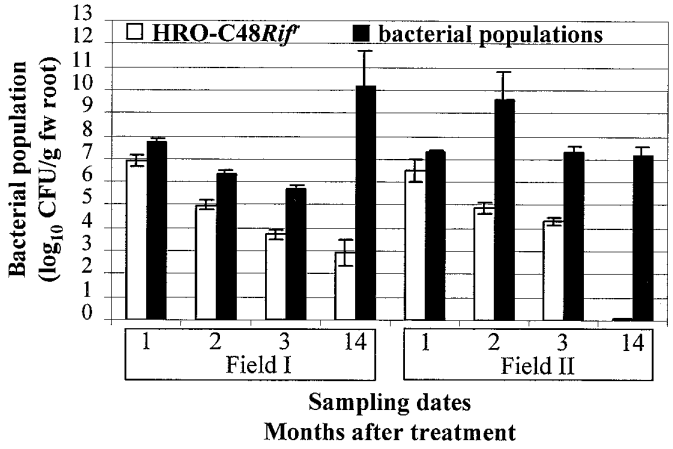

Fig. 3. Mean bacterial plate counts from the rhizosphere of field trial I (Stuthof, 1997 and 1998) and field trial II (Goorstorf, 1998 and 1999). Rhizosphere population densities of the rifampicin-containing agar (nutrient agar, 100 µg/ml of rifampicin) reisolated strain HRO-C48*Rif*^{R} (spontaneous rifampicin resistant mutant) and bacterial populations (CFU on nutrient agar) were determined as Log_{10} CFU per gram of root fresh weight (fw root). Populations at each sampling time (1: young plants, 2: flowering plants, 3: fruiting plants, 4: flowering plants in the second year of harvest) represent the average bacterial population densities of six replicates in Stuthof and three replicates in Goorstorf. Significant differences ($P \leq 0.05$) were determined by Mann-Whitney and are indicated by bars.

The rhizosphere competence of *S. plymuthica* HRO-C48 was demonstrated by reisolation of the rifampicin-resistant mutant from the rhizosphere at levels of approximately 3 to 7 log_{10} CFU/g of root over a period of 14 months under field conditions. Kloepper et al. (19) showed colonization by *S. plymuthica* strain 2-67 of the cucumber rhizosphere over a period of 21 days to a final population density of 4 to 5 log_{10} CFU/g of root, and also inside the root at levels of about 2 log_{10} CFU/g of root. Endophytic colonization of the root has not been investigated for *S. plymuthica* HRO-C48. Scher et al. (30) defined colonizers of corn roots as bacteria that attain CFU of more than 3.5 CFU/g log_{10}. Weller (34) suggested that, as far as introduced bacteria are concerned, a root colonizer is a bacterium that becomes distributed along the root in natural soil, propagates, and survives for several weeks in the presence of competition from the indigenous rhizosphere microflora.

In our study, a high biocontrol activity was correlated with a high yield enhancement. Increases in yield were not observed when disease symptoms were absent (e.g., in field trial II, 1998, Goorstorf). In field trials I (1997, Stuthof) and III (1999, Bühl), disease incidence was high and *S. plymuthica* HRO-C48 was able to reduce disease symptoms and to enhance the yield. In general, the incidence of Verticillium wilt is influenced by the initial inoculum density of microsclerotia (16); the cropping history of the field site (16); the abiotic conditions, such as weather conditions and stress (9); soil parameter (14); and the presence of other pathogens, such as nematodes (28). In our study, different levels of microsclerotia in the soils of the field trials occurred. In field trial I (1997, Stuthof), the number of microsclerotia was higher (40 microsclerotia/g of soil) compared with field trial II (1998, Goorstorf; 21 microsclerotia/g of soil). Symptoms of *V. dahliae* were monitored only in field trial I. However, mean values from 1 to 2 microsclerotia of the fungus per gram of

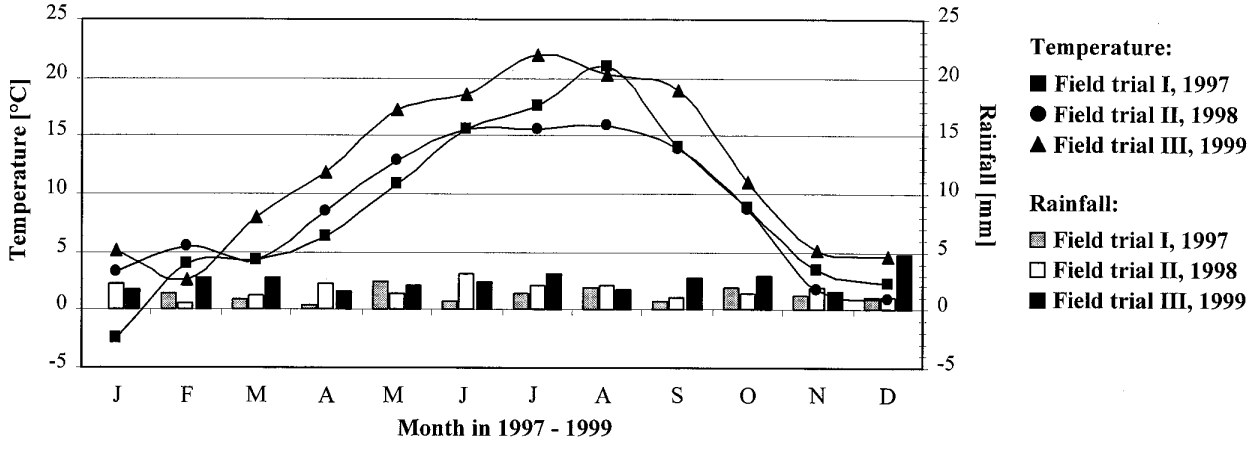

Fig. 4. Monthly mean air temperature (°C) and total rainfall for field trial I (1997, Stuthof), field trial II (1998, Goorstorf), and field trial III (1999, Bühl).

soil have been demonstrated to infect the susceptible strawberry cv. Elsanta under optimal conditions for an infection of the fungus (16). The cropping history of a site is also a good indicator of wilt risk (16). Plants that increase the soil inoculum of *V. dahliae,* such as potato, cotton, brassica crops, sugar beet, and miscellaneous vegetables, enhance the potential risk of infection. Field trial I was planted after oilseed rape, a host of *V. dahliae* which was responsible for the high inoculum level of *V. dahliae* (36). In contrast, field trial II was planted on a field formerly used for barley, a plant that does not host *Verticillium* spp. Weather conditions also had an influence on the field trials. For example, the infection data of field trial I reflect the fact that years with a warm, dry summer are known to favor wilt in strawberry. Soil parameters (sand, nutrients) in the different trials were very similar and we do not consider them a factor.

The plant growth promoting ability of *S. plymuthica* was observed in phytochambers and was confirmed in greenhouse and field trials. Treatment with *S. plymuthica* resulted in an increase of the number of buds, blossoms, and fruit. In addition, intense root branching for plants treated with *S. plymuthica* HRO-C48 was observed in the greenhouse and field trials (*data not shown*). The in vitro test in phytochambers is an easy and fast assay to test the plant growth-promoting potential and is ideal to screen a large number of isolates. The greenhouse trials using soil artificially and naturally infested by *S. plymuthica* are suitable to check the efficacy under defined abiotic and biotic conditions. However, the success of biological approaches to control plant diseases must ultimately be judged by their performance under field conditions. In our field trials, *S. plymuthica* HRO-C48 showed efficacy in controlling wilt and root rot of strawberry caused by *V. dahliae* and *P. cactorum*.

In this study, the strategy for effective selection and evaluation of a potent biocontrol agent was successful. On the basis of the presented results, it was possible to patent *S. plymuthica* HRO-C48 as a biological control agent (8). The results described here are promising for commercial application of *S. plymuthica* strain HRO-C48. The development of a commercial formulation of *S. plymuthica* HRO-C48 is in progress and, in the near future, RhizoStar will be commercially available from PROPHYTA GmbH (Malchow/Poel, Germany). Artificial inoculation with *S. plymuthica* offers a possibility for controlling Verticillium wilt and Phytophthora root rot that can be integrated into the agricultural production process and is sustainable and environmentally friendly.

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