**Biocontrol of *Pythium* in the pea rhizosphere by antifungal metabolite producing and non producing *Pseudomonas* strains.**

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Abbreviated title: *Pseudomonas* and biocontrol

Number of text pages: 25

Number of tables: 5

Number of figures: 0

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**ABSTRACT**

Four well-described strains of *Pseudomonas fluorescens* were assessed for their effect upon pea growth and their antagonistic activity against large *Pythium ultimum* inocula. The effect of *Pseudomonas* strains upon the indigenous soil microflora, soil enzyme activities and plant growth in the presence and absence of *Pythium* is assessed. *Pythium* inoculation reduced the shoot and root weights, root length, and the number of lateral roots. The effect of *Pythium* was reduced by the *Pseudomonas* strains as follows: F113, SBW25 and CHAO increased the shoot weights (by 20%, 22% and 35% respectively); strains Q2-87, SBW25 and CHAO increased root weights (14%, 14% and 52%); Strains SBW25 and CHAO increased the root lengths (19% and 69%), and increased the number of lateral roots (14% and 29%). All the *Pseudomonas* strains reduced the number of lesions and the root and soil *Pythium* populations, whilst SBW25 and CHAO increased the number of lateral roots. *Pythium* inoculation increased root and soil microbial populations but the magnitude of this effect was *Pseudomonas* strain specific. *Pythium* increased the activity of C, N and P cycle enzymes, whilst the *Pseudomonas* strains reduced this effect, indicating reduced plant damage. Overall, strains SBW25 and CHAO had the greatest beneficial characteristics as these strains produced the greatest reductions in the side effects of *Pythium* infection (microbial populations and enzyme activities) and resulted in significantly improved plant growth. Surprisingly strain SBW25 does not produce antifungal metabolites, and its biocontrol activity was related to a greater colonisation ability in the rhizosphere.

## INTRODUCTION

Modern agriculture is highly dependent on chemical pesticides, in order to control plant pathogens. Fungicides and fumigants commonly have drastic effects on the soil biota, as they are intentionally applied at much higher rates than herbicides and insecticides (Fraser 1994). These methods are time-consuming and uneconomical, pollute the atmosphere, and can be environmentally harmful as the chemicals may build up in the soil (Nannipieri 1994). Furthermore, the repeated use of such chemicals has encouraged the development of resistance among the target organisms (Goldman *et al* 1994). This has resulted in the use of ever-increasing amounts of pesticides and has prompted the search for new strategies of pest control to reduce or eliminate the use of pesticides (Cook and Granados 1991, Lorito *et al* 1994). For instance, integration of biocontrol agents with reduced doses of chemical agents has a potential for controlling plant pathogens with minimal impact on the environment (Chet and Inbar 1994).

A number of *Pseudomonas* strains have been intensively studied as possible biocontrol agents of soil borne fungal diseases. Among them are *Ps. fluorescens* strain F113 (Fenton *et al* 1992), *Ps. fluorescens* strain CHAO (Duffy and Defago 1997, Schnider *et al* 1995) and *Ps. fluorescens* strain Q2-87 (Mazzola *et al* 1995). The biocontrol effect of all three of these strains is related in part to their ability to produce the antifungal compound 2,4-diacetylphloroglucinol (Phl) (Keel *et al* 1992, Shanahan *et al* 1992a, Vincent *et al* 1991), while strain CHAO also produces a second antibiotic, pyoluteorin (Natsch *et al* 1998).

Phl is a key factor in the biocontrol of fungal diseases such as damping off of sugarbeet (Fenton *et al* 1992), take-all of wheat and black rot of tobacco (Keel *et al* 1992). Phl production in soil and the rhizosphere by *Pseudomonas* has been detected by a number of researchers (Keel *et al* 1992, Shanahan *et al* 1992b). *P. ultimum* causes damping off of pea seedlings, and the Phl

producing strains inhibit *Pythium* growth in plate assays (Fenton *et al* 1992), deletion of the biosynthetic locus for Phl production showed that that the ability to produce Phl in strain F113 was responsible for the biocontrol properties of this strain but did not contribute to its rhizosphere competence (Carroll *et al* 1995, Fenton *et al* 1992).

*Pseudomonas fluorescens* strain SBW25 is also well described in the literature (De Leij *et al* 1995, De Leij *et al* 1998, Naseby and Lynch 1998a, b) does not produce the antibiotics pyoluteorin or Phl, and does not inhibit *Pythium* growth in plate assays. The three Phl producing strains of *Ps. fluorescens* and strain SBW25 are here compared for their effect upon pea growth and their antagonistic activity against large *P. ultimum* inocula. The effects of the *Pseudomonas* inocula upon the indigenous soil microflora and soil enzyme activities in the presence and absence of *Pythium* are also assessed.

## MATERIALS AND METHODS

### Soil description.

The soil used was sandy loam of the Holiday Hills series, taken from Merrist Wood Agricultural College (Surrey), and had been under permanent pasture for at least 15 years. The analysis of the soil, conducted at the University of Surrey, was pH 5.4, particle ratio 10:9:81 clay: silt: sand respectively, and organic matter content 1.6 % by weight. The total NPK contents by weight were 0.124%, 0.033% and 0.861% respectively.

### Microbial strains and treatments.

Four well-known strains of *Pseudomonas fluorescens* were used. Strain SBW25 EeZY6KX, was isolated from the phytosphere of sugar beet, modified to confer the lacZY, xylE and $Km^r$ marker genes (Bailey *et al* 1995). Strain F113, which produces the antibiotic 2,4 diacetylphloroglucinol (Phl), was marked with a lacZY gene cassette (Fenton *et al* 1992). CHAO, a rifampicin resistant strain, which produces the antibiotics Phl and pyoluteorin (Maurhofer *et al* 1995), and a rif resistant strain of Q2-87 which produces Phl (Mazzola *et al* 1995).

The bacteria were grown on full strength, tryptone soya agar (Oxoid) for 3 days at 30$^o$C. The bacteria were suspended in 10 ml of sterile quarter strength Ringer's solution using disposable plastic plate spreaders to scrape off the bacterial mat, and colony forming units (c.f.u.) were determined by direct counting and spread plates. Control plates (without bacteria) were also flooded with quarter strength Ringers solution and surface scraped with spreaders. The resulting suspensions containing $6x10^9$ c.f.u./ml of the bacteria were subsequently used to imbibe pea seeds (*Pisium sativum* var. Montana), at a ratio of one seed per mL, for 8 hours (stirred every 30 minutes) prior to planting, resulting in $2.8 \times 10^8 \pm 0.4 \times 10^8$ c.f.u. per pea seed. No significant differences in inoculation potential between strains were observed.

*Pythium ultimum* (IMI 308273) was obtained from CABI Bioscience. Material from stock cultures was grown on plates of potato dextrose agar (PDA) at $25^0$C for 3 days (primary plates). Four 5mm disks were cut and placed in a flask containing: 95 g of sand, 5 g of organically grown processed oats and 20 ml of distilled water, all previously autoclaved twice. The flasks were incubated for 3 weeks at $25^0$C and mixed at 7 day intervals. After incubation the *Pythium* media containing both oospores and hyphae was homogenised in a blender and mixed with coarsely sieved soil at a concentration of 3% w/w, resulting in log 4.6 cfu per g soil (determined by spread plates).

**Experimental design.**

*Pythium* inoculated or uninoculated soil (150 g) was placed in experimental microcosms consisting of 210 mm high acetate cylinders, slotted between the top and base of plastic 90 mm diameter Petri dishes creating semi-enclosed microcosms (Naseby and Lynch 1998a). Each microcosm consisted of eight imbibed seeds, planted at a depth of approximately 1 cm below the soil surface. Each *Pseudomonas* treatment (microcosm) and controls were replicated five times in the presence and absence of *Pythium*. Twenty-five ml of water was added to each microcosm before all microcosms were placed in a random design into a growth chamber (Vindon Scientific) set at a 16 hour photoperiod with a day/night temperature regime of $21^oC/15^oC$ respectively. The relative humidity was maintained at 70% and the light intensity was 10,000 lux at shelf level.

**Sampling and analysis.**

After 21 days of growth, the plants were harvested, the number of plants emerged were counted and the individual plant shoot and root weights measured. Subsequently the number of lateral roots and lesions along with the root lengths were measured for 5 plants per microcosm. Rhizosphere soil (closely associated with the plant roots) was collected by shaking soil closely

associated with the roots over a 2 mm sieve and stored at $4^{0}$C until required on the same day. The soil in each group was subsequently assayed for acid and alkaline phosphatase, urease, β-glucosidase, N-acetyl glucosaminidase and aryl sulphatase (Naseby and Lynch 1997).

One gram of pooled fresh root samples, were taken from each microcosm and macerated in 9 ml of sterile quarter strength Ringers solution using a pestle and mortar. One gram of rhizosphere soil from each microcosm was also suspended in 9ml of sterile quarter strength Ringers solution. A ten fold dilution series of each root macerate or soil suspension plated onto 10% malt extract agar containing 100 ppm streptomycin and 50 ppm rose Bengal, incubated at $20^{o}$C for 5 days, was used to enumerate filamentous fungal populations. P1 medium (Katoh and Itoh 1983) was used for the enumeration of indigenous root, fluorescent *Pseudomonas*, after 5 days growth at $25^{o}$C. To enable quantification of introduced *Ps. fluorescens* F113 and SBW25 strains, P1 medium was amended with 50 ppm X-Gal upon which recovered *lac*ZY modified *Pseudomonas* could be identified as blue colonies. Separate P1 plates amended with 50ppm rifampicin were used for the enumeration of the CHAO and Q2-87 strains. Tryptone soya agar (10%) was used for the enumeration of total culturable bacteria and incubated for 7 days at $25^{o}$C. VP agar (Lumsden *et al* 1990), based on potato dextrose agar, was used to enumerate *Pythium* and contained the following supplements: Vancomycin (200 mg $l^{-1}$), pimaricin (10 mg $l^{-1}$), pentachlorobenzene (100 mg $l^{-1}$), streptomycin (50 mg $l^{-1}$) and rose bengal (2.5 mg $l^{-1}$). *Pythium* populations were enumerated after 3 days growth at $20^{o}$C.

**Statistical analysis.**

Data were analysed using SPSS for Windows (SPSS inc.) by means of a one-way ANOVA and subsequently differences among treatments (multiple comparisons) were determined using least significant differences (LSD) between means as the post hoc test. Emergence and shoot/root ratio data were transformed into $log^{it}$ before statistical analyses as above.

## RESULTS

### Plant growth in the presence of *Pythium*.

*Pythium* inoculation reduced the emergence of pea seedlings (Table 1) and this effect was significantly ($P$<0.05) suppressed by *Pseudomonas* strains SBW25 and F113. Strains F113, SBW25 and CHAO significantly ($P$<0.05) increased the wet shoot weights by 22%, 35% and 20% respectively (Table 1). *Pseudomonas* strains Q2-87, SBW25 and CHAO resulted in significantly ($P$<0.05) greater wet root weights (by 14%, 14% and 52% respectively) than the *Pythium* control (Table 1). Only CHAO significantly ($P$<0.05) affected the shoot/root ratio, resulting in a significantly lower ratio than *Pythium* control, F113 and SBW25 treatments (Table 1).

*Pythium* inoculation significantly reduced the root length and the number of lateral roots (Table 2). Inoculation with all the *Pseudomonas* strains significantly ($P$<0.05) reduced the number of lesions caused by *Pythium*, with strain CHAO resulting in the greatest reduction. Strains SBW25 and CHAO significantly ($P$<0.05) increased the root length by 19% and 69% respectively, and the number of lateral roots by 14% and 29% respectively.

### Plant growth in the absence of *Pythium.*

Strain CHAO resulted in a significantly ($P$<0.05) lower pea emergence than strains F113 and Q2-87, whilst none of the inocula significantly affected the shoot weight and only CHAO increased the root weight in comparison to the control and Q2-87 treatments (Table 1). *Pseudomonas* strains F113 and CHAO resulted in significantly lower shoot/root ratios than the control and SBW25.

Inoculation with *Pseudomonas* strain Q2-87 significantly reduced the root length in comparison with all the other treatments, whilst strains Q2-87, F113 and CHAO reduced the number of lateral roots in relation to the control and SBW25 treatments (Table 2).

**Microbial populations.**

*Pythium* inoculation significantly increased the root and rhizosphere soil bacterial populations (Table 3). *Pseudomonas* strains Q2-87 and F113 significantly increased the root total bacterial populations in the absence of *Pythium,* whilst strain CHAO resulted in a smaller soil bacterial population than the control. All the *Pseudomonas* strains resulted in significantly lower soil and root bacterial populations in the presence of *Pythium* than the *Pythium* control, with strain CHAO resulting in the lowest bacterial populations. *Pythium* inoculation significantly increased the indigenous *Pseudomonas* populations and this effect was significantly reduced by inoculation with strains SBW25 and CHAO (Table 3). Strain SBW25 established the greatest population (introduced *Pseudomonas*) in the absence of *Pythium*, whilst strain CHAO also colonised at significantly greater levels than strains F113 and Q2-87. In the presence of *Pythium,* a significantly lower population of strain CHAO than of the other three strains was found. The total *Pseudomonas* population in the absence of *Pythium* was increased by the *Pseudomonas* inocula in the following order SBW25 > CHAO > F113, Q2-87 > control. *Pythium* inoculation significantly increased the total *Pseudomonas* populations, and was increased further by the inoculation of strains Q2-87, F113 and SBW25.

The root fungal populations in the absence of *Pythium* (Table 4) were significantly greater with the inoculation of *Pseudomonas* strains Q2-87, F113 and CHAO than the control. Strains F113 and CHAO significantly reduced the soil fungal population in comparison to the control. *Pythium* inoculation alone, significantly increased ($P<0.05$) the root and rhizosphere soil fungal populations (Table 4). Strain CHAO significantly reduced and strain Q2-87 significantly

increased (*P*<0.05) the root fungal populations in relation to the *Pythium* control and the other *Pseudomonas* treatments (Table 4). All the *Pseudomonas* inocula significantly reduced the soil fungal populations in relation to the *Pythium* control to varying degrees, with strain CHAO resulting in significantly (*P*<0.05) the lowest soil fungal population.

*Pseudomonas fluorescens* CHAO reduced the root *Pythium* population to undetectable levels whilst the other *Pseudomonas* inocula also significantly reduced the root *Pythium* population by varying degrees (Table 4). All the *Pseudomonas* strains significantly reduced the soil *Pythium* populations, with SBW25 resulting in the lowest population. Root and soil *Pythium* populations were not detected in treatments without *Pythium* inoculation.

**Soil enzyme activities.**

Strains Q2-87, F113 and CHAO significantly increased acid phosphatase, sulphatase and urease activities with respect to the control in non *Pythium* soil (Table 5). Strains Q2-87 and F113 also significantly increased (*P*<0.05) the alkaline phosphatase activity in the soil without *Pythium*. Strains Q2-87, F113 and CHAO significantly reduced (*P*<0.05) the β-glucosidase and NAGase activities in soil without *Pythium* inoculation.

There were significantly higher (*P*<0.05) enzyme activities in soil infected with *Pythium* than non infected soil (Table 5). Inoculation with *Pseudomonas* strains significantly reduced (*P*<0.05) the effect of *Pythium* as follows. All four *Pseudomonas* strains reduced the sulphatase and urease activities. Strains Q2-87, SBW25 and CHAO reduced the alkaline phosphatase activity. Strains SBW25 and CHAO reduced the β-glucosidase activity and only strain CHAO reduced the NAGase activity. Only strain Q2-87 significantly (*P*<0.05) increased the acid phosphatase activity, relative to the *Pythium* control.

## DISCUSSION

### Plant growth.

The lower emergence rate of seedlings inoculated with strain CHAO may be due to a slight deleterious effect sometimes found with this strain, which is related to the production of the antibiotics, Plt and Phl (Maurhofer *et al* 1995). *Pythium* ultimum is most destructive at the seedling stage (Kommedahl *et al* 1981) and reduced pea emergence in this study. This effect was, however, suppressed by *Pseudomonas* strains SBW25 and F113, in concordance with the suggestions of Cook (Cook 1994) who stated that the ability of *Pythium* to rapidly colonise the host plant before other microorganisms is an essential part of their pathogenicity.

*Pseudomonas* strains SBW25 and CHAO had the greatest overall effects in the presence of *Pythium*, resulting in increased plant weights, root lengths and lateral roots, whereas the effects of strains F113 and Q2-87 were sporadic. The improvement in plant growth was related to a reduction in the root *Pythium* population to undetectable levels with the inoculation of CHAO, which is corroborated by an 80% reduction in the number of root lesions. The other strains also reduced the root *Pythium* populations and all four *Pseudomonas* strains reduced the soil *Pythium* populations and reduced the number of root lesions. Therefore, the smaller improvements in plant growth with Q2-87 and F113 are related to the large reduction in the soil *Pythium* population rather than the root *Pythium* population and a greater reduction in the number of lesions than strains F113 and Q2-87.

The basis of the biocontrol activity of three of the strains (Q2-87, F113 and CHAO) is antibiotic production, however, strains Q2-87 and F113 only produce Phl (Keel *et al* 1996), whereas CHAO also produces Plt (Natsch *et al* 1997). Therefore, as the performance of CHAO was much greater than F113 and Q2-87, the combination of the two antibiotics is more than

additive on effectiveness of CHAO as a biocontrol agent. *Pseudomonas* strain SBW25 had a greater plant protective effect than Q2-87 and F113, yet this strain does not produce Phl or Plt and did not produce other soluble metabolites capable of significantly retarding the growth of *Pythium* in place assays. Therefore, the biocontrol activity of this strain is not related to antibiotic production, however, it may be related to the ability of this strain to colonise the rhizosphere at much higher levels than some other *Pseudomonas* strains (Naseby and Lynch 1998b) including the other three strains described here (Table 4

**Microbial Populations.**

The Q2-87, F113 and CHAO strains increased the root fungal populations whereas the F113 and CHAO strains reduced the rhizosphere soil fungal populations with respect to the control. Similar results were found with the root and rhizosphere soil bacterial populations with the Q2-87 and F113 treatments increasing root bacteria and the CHAO treatment reducing soil bacterial populations. These results are therefore contradictory in nature, however the explanation may be linked to an increase in root exudate/leakage with the inoculation of Phl producing strains (Naseby *et al* 1999) which would support a greater root population.

The reduced populations in the rhizosphere soil may be linked to the antibiotic production which is supported by Short (Short *et al* 1990) who found that soil fungal populations were suppressed by a strain of *Pseudomonas putida* inoculated into soil. Volatile organic compounds produced by various soil bacteria have been shown to mediate effects in fungi (Mackie and Wheatley 1999), which appeared to be species-specific, with each fungus responding uniquely to the products of each of the bacterial cultures.

Other studies investigating the effect of Phl producers on soil or root microbial activities have found similar effects. *Pseudomonas fluorescens* CHAO and a Phl and Pyoluteorin over-producing

derivative were found to have a similar but transient increase in the metabolic activity of resident root bacterial community (Natsch *et al* 1998). Furthermore, it has been shown that the F113 strain significantly reduced the soil microbial activity (Brimecombe *et al* 1998), therefore, it follows that the CHAO strain had greater effect on the soil bacterial population due to the production of two antibiotics rather than one. However, it must be noted that none of these previous studies investigated the effect of Phl producers on root and soil microbial populations concurrently.

The increased root and rhizosphere soil bacterial and fungal populations in the presence of *Pythium* are probably due to the pathogenic effect of the pathogen causing nutrient leakage from the root, as has been shown previously (Naseby *et al* 1999 and 2000). The *Pseudomonas* strains, in general, reduced this effect, which is due to the reduction in the effect of the pathogen on plant growth, indicating that the pathogen caused less root damage in the presence of the *Pseudomonas* inocula. It therefore follows that the greatest reductions in the microbial populations are found with the inoculation of *Pseudomonas* strains which offered the greatest protection to plant growth, i.e., strain CHAO and to a lesser extent strain SBW25. This is supported by the fact that only strains SBW25 and CHAO significantly reduced the indigenous *Pseudomonas* population relative to the *Pythium* control, in *Pythium* treated soil.

The additive effect of the introduced *Pseudomonas* was responsible for the increased total *Pseudomonas* populations with all four *Pseudomonas* inocula. The greater increases in the total *Pseudomonas* populations found with the superior colonising abilities of strains SBW25 and CHAO support this. The additive effect was also found in the *Pythium* infected soil for all four *Pseudomonas* inocula. This includes strain CHAO which resulted in a smaller indigenous population but a similar total population. The high colonising ability of strain SBW25 (Naseby and Lynch 1998a, b) accounts for the greater total *Pseudomonas* population than the *Pythium*

control, as the SBW25 treatment also resulted in a smaller indigenous *Pseudomonas* population than the *Pythium* control.

**Soil enzyme activities.**

Measurement of soil enzyme activities may be useful for gaining a greater understanding of the nature of perturbations caused to ecosystem function and has been used as an indicator of the effect of microbial inoculation (Naseby and Lynch 1998b, Naseby *et al* 1999).

The greater acid and alkaline phosphatase, aryl sulphatase and urease activities with Q2-87, F113 and to a lesser extent CHAO, but not the SBW25 treatments infers that this is related to the production of Phl. This is corroborated by the fact that Naseby and Lynch (1998b) found a similar impact on soil enzyme activities with the inoculation of strain F113, which was not found with a Tn5 mutated Phl negative derivative of strain F113. Increased available inorganic soluble phosphate is known to have an inverse effect on soil phosphatase activity (Tabatabai 1982, Tadano *et al* 1993) and similar trends occur in sulphatase activity in relation to sulphate availability. If this theory is correct, the Q2-87, F113 and CHAO and strains must have caused a decrease in the available phosphate and sulphate, thus causing an overall increase in phosphatase and sulphatase activities. The decrease in available P and S may have been caused by an increase in the amount of available carbon in the rhizosphere as was indicated by a reduction in C cycle enzyme activities. This is supported by the fact that increased available carbon in the rhizosphere of F113 inoculated pea plants has been shown previously (Naseby *et al* 1999). Therefore, an increase in the ratio of C to available P and S leads to an increase in microbial P and S demand and increased phosphatase and sulphatase activities and reduced C cycle activities.

The general increase in enzyme activities found in the presence of *Pythium* therefore indicate a dramatic increase in C and nutrient leakage from roots due to root damage. In conditions of high

C availability, such as root leakage, P is a more limiting nutrient and demand increases resulting in an increase in phosphatase activity (Naseby and Lynch 1998, Naseby *et al* 1999). Urease activity (N cycle) was also increased by *Pythium* infection, which again indicates increased C availability as shown by Naseby *et al* (2000).

All the strains inoculated reduced the effect of the *Pythium* on soil enzyme activities by varying degrees. All four strains reduced the sulphatase and urease activities suggesting a reduction in C leakage from the root. A further indication is the reduction in alkaline phosphatase activity with strains Q2-87, SBW25 and CHAO compared to the *Pythium* control. C cycle enzyme activities (NAGase and β-glucosidase) were also reduced by strains SBW25 and CHAO in relation to the *Pythium* control, which again indicates a comparative reduction in root damage. The reduction in a number of enzyme activities, especially by strains SBW25 and CHAO, therefore indicates a reduction in plant damage and subsequent C leakage caused by *Pythium,* and is also related to increases in the plant growth described earlier with these strains. This is supported by the fact that inoculation with strains Q2-87 and F113 resulted in less reductions in enzyme activities in relation to the *Pythium* control than SBW25 and CHAO and these strains offered less protection to the plant as shown by the plant growth measurements.

Overall, strain CHAO had the greatest beneficial characteristics as it consistently reduced the damage caused to the pea plants by *P. ultimum*, and reduced the population of the pathogen on the root to undetectable levels. However, the slight detrimental effect of CHAO on pea emergence in the absence of *Pythium* may reduce the value of this strain for use in the field and further examination is needed. Strain SBW25 surprisingly performed better as a biocontrol agent than the Phl producing strains Q2-87 and F113 did. Therefore strain SBW25, on this evidence, warrants further investigation as a possible biocontrol agent.

## ACKNOWLEDGMENTS

This work was supported by the EU Biotechnology Programme contract no. BIO4-CT96-0027, the IMPACT project which incorporates 17 European partners.

We thank Fergal O'Gara of University College Cork for providing the F113 construct; Mark Bailey of IVEM, Oxford for providing the SBW25 construct; Genevieve Defago of the Swiss Federal Institute of Technology for provision of the CHAO strain and Linda Thomashow of Washington State University for providing the Q2-87 strain.

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TABLE 1. Pea emergence, shoot and root weights as affected by *Pseudomonas* inocula and *Pythium ultimum.*

| Treatment[1] | Plant growth[2] | Emergence[3] | Shoot[3] (g) | Root[3] (g) | s/rRatio[3] |
|---|---|---|---|---|---|
| Soil without *Pythium* | Control | $0.93^{bc}$ | $0.90^{c}$ | $0.92^{c}$ | $0.98^{bc}$ |
| | SBW25 | $0.93^{bc}$ | $0.94^{c}$ | $0.96^{cd}$ | $0.98^{bc}$ |
| | Q2-87 | $1.0^{c}$ | $0.85^{bc}$ | $0.92^{c}$ | $0.92^{ab}$ |
| | CHAO | $0.88^{ab}$ | $0.90^{c}$ | $1.06^{d}$ | $0.84^{a}$ |
| | F113 | $0.98^{c}$ | $0.86^{bc}$ | $1.03^{cd}$ | $0.84^{a}$ |
| Soil treated with *Pythium* | Control | $0.8^{a}$ | $0.69^{a}$ | $0.66^{a}$ | $1.05^{cd}$ |
| | SBW25 | $0.95^{bc}$ | $0.84^{bc}$ | $0.75^{b}$ | $1.12^{d}$ |
| | Q2-87 | $0.85^{ab}$ | $0.76^{ab}$ | $0.75^{b}$ | $1.01^{bc}$ |
| | CHAO | $0.85^{ab}$ | $0.93^{c}$ | $1.00^{cd}$ | $0.93^{ab}$ |
| | F113 | $0.93^{bc}$ | $0.83^{bc}$ | $0.74^{ab}$ | $1.12^{d}$ |

[1] Treatments; control, no inocula; SBW25, inoculated with *lac*ZY marked *Ps. fluorescens* SBW25 EeZY6KX; Q2-87, inoculated with rif marked *P fluorescens* Q2-87; CHAO, inoculated with rif marked *P fluorescens* CHAO; F113, inoculated with *lac*ZY marked Phl+ *Ps. fluorescens* F113.

[2] Emergence, proportion of seedlings emerged five days after sowing; shoot, mean shoot weight; root, mean root weight; s/r ratio, mean ratio of shoot weight to root weight.

[3] Letters, within a column, indicate significant differences at $p<0.05$ level. For emergence the mean of five microcosms is given (n=5). For shoot, root and s/r ratio, n is proportional to the emergence to a maximum of 40.

TABLE 2. Mean root length and number of lateral roots and lesions per root system as affected by *Pseudomonas* inocula and *Pythium ultimum*.

| Treatment[1] | Root measurements[2] | Root length[3] (cm) | Lateral roots[3] | Lesions[3] |
|---|---|---|---|---|
| Soil without *Pythium* | Control | 24.23[c] | 23.45[de] | ND |
| | SBW25 | 23.27[c] | 23.33[de] | ND |
| | Q2-87 | 19.37[b] | 20.73[bc] | ND |
| | CHAO | 23.37[c] | 20.65[bc] | ND |
| | F113 | 24.67[c] | 20.87[bc] | ND |
| Soil treated with *Pythium* | Control | 14.83[a] | 19.33[ab] | 17.05[c] |
| | SBW25 | 17.7[b] | 21.9[cd] | 12.28[b] |
| | Q2-87 | 15.18[a] | 18.55[a] | 13.3[b] |
| | CHAO | 25[c] | 25[e] | 3.6[a] |
| | F113 | 16.85[ab] | 19.5[ab] | 14.2[b] |

[1] Treatments; control, no inocula; SBW25, inoculated with *lac*ZY marked *Ps. fluorescens* SBW25 EeZY6KX; Q2-87, inoculated with rif marked *P fluorescens* Q2-87; CHAO, inoculated with rif marked *P fluorescens* CHAO; F113, inoculated with *lac*ZY marked Phl+ *Ps. fluorescens* F113.

[2] Lateral roots, mean number of lateral roots per root system; lesions, mean no. lesions per root system; ND, none detected.

[3] Letters, within a colunm, indicate significant differences at $p<0.05$ level, five roots per microcosm were measured (n=25).

TABLE 3. $Log_{10}$ bacterial populations as affected by *Pseudomonas* inocula and *Pythium ultimum*.

| Treatment[1] | Bacteria[2] | Root bact[3] | Soil bact[3] | Ind pseu[3] | Int Pseu[3] | Tot Pseu[3] |
|---|---|---|---|---|---|---|
| Soil without *Pythium* | Control | 7.36[a] | 7.86[b] | 6.29[ab] | NA | 6.29[a] |
| | SBW25 | 7.38[a] | 7.82[b] | 6.15[a] | 6.62[c] | 6.75[d] |
| | Q2-87 | 7.58[b] | 7.79[ab] | 6.18[a] | 6.14[a] | 6.46[b] |
| | CHAO | 7.35[a] | 7.64[a] | 6.16[a] | 6.41[b] | 6.6[c] |
| | F113 | 7.56[b] | 7.78[ab] | 6.18[a] | 6.12[a] | 6.45[b] |
| Soil treated with *Pythium* | Control | 8.45[e] | 8.49[d] | 6.62[c] | NA | 6.62[c] |
| | SBW25 | 8.16[d] | 7.87[b] | 6.44[b] | 6.73[c] | 6.91[e] |
| | Q2-87 | 8.19[d] | 8.05[c] | 6.71[c] | 6.63[c] | 6.97[e] |
| | CHAO | 7.92[c] | 7.71[a] | 6.25[a] | 6.48[b] | 6.68[cd] |
| | F113 | 8.24[d] | 8.11[c] | 6.74[c] | 6.68[c] | 7.01[e] |

[1] Treatments; control, no inocula; SBW25, inoculated with *lac*ZY marked *Ps. fluorescens* SBW25 EeZY6KX; Q2-87, inoculated with rif marked *P fluorescens* Q2-87; CHAO, inoculated with rif marked *P fluorescens* CHAO; F113, inoculated with *lac*ZY marked Phl+ *Ps. fluorescens* F113.

[2] Root bact, root bacteria/g root; Soil bact, soil bacteria/g soil; ind pseu, indigenous fluorescent pseudomonads/g root; int pseu, introduced fluorescent pseudomonads/g root; tot pseu, total fluorescent pseudomonads/g root.

[3] Letters, within a column, indicate significant differences at $p<0.05$ level, (n=5).

TABLE 4. $\text{Log}_{10}$ fungal populations as affected by *Pseudomonas* inocula and *Pythium ultimum*.

| Treatment[1] | Fungi[2] | Root fungi[3] | Soil Fungi[3] | Root Pythium[3] | Soil Pythium[3] |
|---|---|---|---|---|---|
| Soil without Pythium | Control | 3.08[a] | 4.56[c] | ND | ND |
| | SBW25 | 3.08[a] | 4.58[c] | ND | ND |
| | Q2-87 | 3.38[b] | 4.42[bc] | ND | ND |
| | CHAO | 3.3[b] | 4.35[b] | ND | ND |
| | F113 | 3.3[b] | 4.37[b] | ND | ND |
| Soil treated with *Pythium* | Control | 3.6[c] | 5.09[e] | 4.1[d] | 4.55[d] |
| | SBW25 | 3.6[c] | 4.41[bc] | 3.91[c] | 3.87[a] |
| | Q2-87 | 3.9[d] | 4.58[c] | 3.62[b] | 4.05[ab] |
| | CHAO | 3.3[b] | 4.08[a] | N[D] | 4.21[bc] |
| | F113 | 3.62[c] | 4.86[d] | 3.34[a] | 4.31[c] |

[1] Treatments; control, no inocula; SBW25, inoculated with *lac*ZY marked *Ps. fluorescens* SBW25 EeZY6KX; Q2-87, inoculated with rif marked *P fluorescens* Q2-87; CHAO, inoculated with rif marked *P fluorescens* CHAO; F113, inoculated with *lac*ZY marked Phl+ *Ps. fluorescens* F113.

[2] Root Fungi, root fungi/g root; Soil Fungi, soil fungi/g soil; Root *Pythium, Pythium/g root;* Soil *Pythium, Pythium/g* soil; ND, Not detected.

[3] Letters, within a column, indicate significant differences at $p<0.05$ level, (n=5).

TABLE 5. Soil enzyme activities in the rhizosphere of pea plants inoculated with *Pseudomonas* and *Pythium ultimum.* n=5

| Treatment[1] | Enzyme[2] | Acid phos[3] | Alk phos[3] | Sulphatase[3] | Urease[3] | β-gluc[3] | NAGase[3] |
|---|---|---|---|---|---|---|---|
| Soil without Pythium | Control | 1.53[a] | 9.57[a] | 0.078[a] | 1.41[a] | 0.61[bc] | 0.32[b] |
| | SBW25 | 1.85[ab] | 9.33[a] | 0.075[a] | 1.17[a] | 0.54[ab] | 0.33[b] |
| | Q2-87 | 3.62[cd] | 10.25[b] | 0.107[bc] | 2.15[bc] | 0.37[a] | 0.21[a] |
| | CHAO | 1.92[b] | 9.94[ab] | 0.099[b] | 1.97[b] | 0.42[a] | 0.24[a] |
| | F113 | 3.27[c] | 10.27[b] | 0.099[b] | 2.08[b] | 0.41[a] | 0.20[a] |
| Soil with *Pythium* | Control | 4.02[d] | 13.13[f] | 0.198[e] | 2.59[d] | 1.15[e] | 0.54[d] |
| | SBW25 | 3.61[cd] | 12.04[cd] | 0.139[d] | 2.29[c] | 0.74[c] | 0.44[c] |
| | Q2-87 | 4.88[e] | 12.48[de] | 0.140[d] | 2.32[c] | 1.21[e] | 0.49[cd] |
| | CHAO | 4.12[d] | 11.55[c] | 0.120[c] | 2.14[bc] | 0.96[d] | 0.33[b] |
| | F113 | 3.97[d] | 12.69[ef] | 0.148[d] | 2.34[c] | 1.13[e] | 0.50[cd] |

[1] Treatments; control, no inocula; SBW25, inoculated with *lac*ZY marked *Ps. fluorescens* SBW25 EeZY6KX; Q2-87, inoculated with rif marked *P fluorescens* Q2-87; CHAO, inoculated with rif marked *P fluorescens* CHAO; F113, inoculated with *lac*ZY marked Phl+ *Ps. fluorescens* F113.

[2] Acidphos, acid phosphatase; alkphos, alkaline phosphatase; Sulphatase, Aryl sulphatase; β-gluc, β-glucosidase; NAGase, N-acetyl glucosaminidase. Activities expressed as mg PNP released/h/g soil. Urease activity expressed as g ammonia released/h/g soil.

[3] Letters, within a column, indicate significant differences at $p<0.05$ level, (n=5).