



ANNUAL REPORT TO ACIAR

Increasing Crop Production through Biological Control of Soil-borne Root Diseases



**Program LVR2
Project PN9680
December 2000 to February 2002**

CSIRO Land and Water
China Agricultural University
Chinese Academy of Agricultural Sciences
Zhejiang University
Australian Cotton Research Institute

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SUPPLEMENTARY REPORT TO ACIAR

December 2000 to February 2002

Project extension

to

**Program LWR2
Project PN 9680**

**INCREASING CROP PRODUCTION
THROUGH BIOLOGICAL CONTROL
OF SOIL-BORNE ROOT DISEASES**

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December 2000 to February 2002

LWR2 / PN 9680

Increasing crop production through biological control of soil-borne root diseases.

Research leaders:

Dr M.H. Ryder, CSIRO Land and Water, Glen Osmond, SA

Dr P.R. Harvey, CSIRO Land and Water, Glen Osmond, SA

Prof. Tang Wenhua, Dept of Plant Pathology, China Agricultural University (CAU), Beijing, China

Prof. Zhang Bingxin, Dept of Plant Protection, Zhejiang University (ZU), Hangzhou, Zhejiang China

Dr Yang Hetong, Shandong Academy of Science (SDAS), Jinan, Shandong Province, China

Project timing:

Commencement date: 1/7/97

Completion date: 30/6/00 amended by extension to 30/6/01

Approved total budget:

Total budget: \$768,549

Variations to date: \$ 98,000

Total \$866,549

Income to date: \$866,549

Expenditure to March 2002: \$857,858 + 8,691 committed

Project abstract:

The major aim of the research was to evaluate soil-borne bacteria and fungi that were originally isolated in Australia and in China for their ability to control target root diseases of wheat, cotton and vegetable crops. The tests were done in a variety of field environments including southern and eastern Australia, northern, northwestern and eastern China. Research was also conducted in glasshouses (greenhouses) in both countries. A second aim was to determine the mechanisms by which certain bacterial and fungal biocontrol agents control disease. The ultimate aim was to obtain new effective and reliable biological control treatments that farmers can use to control soil-borne diseases of the target crops. Significant progress was made in the research conducted in this project. A new biocontrol product was taken to preliminary registration and was commercially available for control of wheat diseases in China (1998 and 1999). Forty to 50 tonnes of inoculum product was produced and sold – enough to treat 33,000 ha of wheat. Further commercial outcomes (development of biological controls) are being considered, both in China and Australia. A feature of the project has been the large training component. Australian scientists spent a total of ca. 10 months in China and Chinese scientists were in Australia for a total of more than 4 person years.

Approved budget and expenditure for period being reported (financial years 7/2001-6/2002)

Approved budget: \$98,000

01/02 expenditure: \$89,309 + 8,691 committed

EXECUTIVE SUMMARY
SUPPLEMENTARY REPORT TO ACIAR

November 2000 to February 2002

Extension to PN LWR2/9680

Increasing crop production through biological control of soil-borne root diseases.

Purpose and context of project:

Soil-borne diseases cause economically important yield losses in most major crops. Many diseases cannot be controlled by plant resistance or crop management practices. The development of effective biological controls will allow farmers to increase productivity and / or increase their flexibility in crop management.

The aims of the overall research program were:

- (1) to evaluate soil-borne bacteria and fungi, isolated in Australia and in China, for their ability to control target root diseases of wheat, cotton and vegetable crops. The tests were conducted in both the greenhouse and the field, in both countries.
- (2) to elucidate the mechanisms by which selected bacterial and fungal treatments control disease and enhance plant growth and yield
- (3) the ultimate aim is to obtain effective and reliable biological control treatments that farmers can use to control important soil-borne root diseases of the target crops.

The **specific aims** of the **project extension** were:

- (1) to further evaluate biocontrol for wheat root diseases in Australia, using Chinese formulation methodology (CSIRO Land and Water).
- (2) to use formulations of bacteria to treat damping-off of vegetables in the field in China (Zhejiang University).
- (3) to further test binucleate *Rhizoctonia* for control of cotton diseases in the field in China (China Agricultural University / Shandong Academy of Science).

Collaborating researchers and institutions:

Dr M.H. Ryder, CSIRO Land and Water, Glen Osmond, SA

Dr P.R. Harvey, CSIRO Land and Water, Glen Osmond, SA

Prof. Tang Wenhua, Dept of Plant Pathology, China Agricultural University (CAU), Beijing, China

Prof. Zhang Bingxin, Dept of Plant Protection, Zhejiang University (ZU), Hangzhou, Zhejiang China

Dr Yang Hetong, Shandong Academy of Science, Jinan, Shandong Province, China

Results and implications of the project extension:

1. Good efficacy of binucleate *Rhizoctonia* from CSIRO to control cotton disease and to significantly increase cotton yield in China has been demonstrated in field trials conducted by CAU. This finding can be followed up in both China and Australia with further field trials and potential for commercial development.
2. *Trichoderma* Tk7a from CSIRO, which was commercialized by CAU for the control of wheat diseases in China in 1998 and 1999, now requires re-registration. Concerning the commercial use of Tk7a in China, Prof. Tang (CAU) is negotiating with Linxian Science & Technology Company, Qinhuandao city (located North-east of Beijing). The proposal is for the company to produce small amounts of *Trichoderma* for further field trials. If the company decides to proceed, CAU plans to re-register the organism for commercial use. This process can be facilitated if a grant (applied for) is obtained from AusIndustry. The Beijing-Goettingen

Biotech Company may also consider developing *Trichoderma* agents for commercial use.

3. CSIRO Land and Water completed the testing of wheat disease biocontrol, using CAU formulation methodology to produce *Trichoderma* inoculum, in the 2001 season. Statistically significant yield responses were obtained in a trial in Western Victoria (2001), adding approx 5% yield to a 5.5t/ha crop (worth an extra \$50/ha at \$180 per tonne). Performance was equal to that of chemical fungicides. Results in 2000 at Auburn SA were also promising; with substantial yield increases being recorded with both chemical fungicide and *Trichoderma* treatment.

There is good potential for further development of *Trichoderma* in Australia, and this is likely to be pursued via advertising for expression of interest for commercial development, with a potential for co-investment by the Grains R&D corporation. An AusIndustry grant has been applied for, which, if successful, would enable continued collaboration with researchers in China, with a focus on commercial development.

4. Bacterial strains have been formulated for greenhouse testing by Zhejiang University staff and students. Dr Yang Hetong, Shandong Academy of Science (SDAS), developed the formulation and Rosemary Warren and Paul Harvey (CSIRO) taught the methodology during a visit in November 2001.
5. A substantial number of research reports and papers have been published (see Appendix 4 of the report for details).
6. Continued cooperation between China and Australia:
 - (a) Because of the strong commercial development of biocontrol agents by CAU and SDAS, and the successful use of Chinese formulations in the CSIRO field testing program, there is a good possibility for further commercial development in both countries. Accordingly, a proposal was submitted to AusIndustry for inter-country exchange visits. The success of this proposal will be decided in May 2002. Other Chinese co-operators are also likely to be able to benefit from this continued connection.
 - (b) A proposal to conduct a workshop on crop root disease and their control in NW China is under development by CAU with the assistance of CSIRO, for submission to AusAID. The possible location is Inner Mongolia. If this application can be submitted within the guidelines and the workshop and associated activities can be successfully achieved, this may provide a model that could be adopted in other parts of China.
 - (c) A connection between Zhejiang University and CSIRO continues, on the microbial ecology of biocontrol agents and soil-borne pathogens. (Funding: Chinese National Natural Science Foundation (2001 – 2004).
 - (d) Cooperation between SDAS and CSIRO: exchange visits on the biological control of plant disease and related topics. (Recently funded from China).

3. PROGRESS OF RESEARCH WORK

3.1 Objectives of project

1. To test the effectiveness and reliability of promising biological control agents for take-all and Rhizoctonia diseases of wheat under field conditions.
2. To evaluate biological agents for reliable control of damping-off on Solanaceous and Cucurbitaceous vegetables.
3. To evaluate the potential for biological control of damping-off and Verticillium wilt of cotton.
4. To investigate the biological basis for biocontrol of soil-borne diseases and plant growth promotion.

The **specific aims** of the **project extension** were:

- (1) to further evaluate biocontrol for wheat root diseases in Australia, using Chinese formulation methodology (CSIRO Land and Water).
- (2) to use formulations of bacteria to treat damping-off of vegetables in the field in China (Zhejiang University)
- (3) to further test binucleate Rhizoctonia for control of cotton diseases in the field in China (China Agricultural University / Shandong Academy of Science)

3.2 Research activities

(i) Timetable and personnel:

ZU: staff involved were Prof. Zhang BingXin, Ms Lou Binggan and students.

CAU:

Prof. Tang Wenhua: overall coordination.

Dr Ma Ping (cotton trials, Hebei province), Prof Li Hong-lian (cotton trials, Henan province),

Ms Yan Xioxue (cotton trials, Xinjiang region)

Dr Wang Ye, Dr Zhang Liqun and Dr Yang Hetong (CAU / Shandong Academy of Science)

CSIRO:

Dr M Ryder: Project coordination.

Ms R. Warren, Dr M. Ryder: field trials, 2001.

Wesfarmers Landmark assisted CSIRO in the establishment, maintenance and harvest of field trials.

Dr M. Ryder, Dr P. Harvey: liaison with Chinese co-operators.

International visits:

Dr Ryder, visited China in June 2001 and Dr Harvey and Ms R Warren visited China in October / November 2001.

(ii) Analysis and research methods:

Field trials in Australia (wheat): methods are fully outlined in Appendix 2.

Field trials in China (cotton): the methods are described in Appendix 3.

Formulation of micro-organisms is described in Appendix 2 and Appendix 3.

(iii) Results and Implications:

Objective 1:

Biocontrol of wheat diseases, Australia: CSIRO (Appendixes 1 and 2):

Control of take-all in the field, 2000 (yield data; Appendix 1)

The major disease in 2000 was cereal cyst nematode, which was expected at one site (Auburn, SA), based on the soil DNA test, but not at the other (Peake, SA) because there was no time to have a test done during the site selection process.

The Auburn trial design was factorial having all treatments plus and minus Temik nematicide. Temik reduced CCN damage as expected and increased yield (+10% across all treatments, F. pr. = 0.017). Interestingly, Impact chemical fungicide and *Trichoderma* Tk7a reduced CCN also, and increased yield in the absence of Temik (+24% yield and +18% yield, respectively, F. pr. = 0.057, on crop yields in the range 5 t/ha). Only Impact fungicide gave an additional yield benefit in the presence of Temik, and Impact + Temik gave the best result of all in terms of CCN control and yield. This result may indicate an ability of chemical and biological fungicides to suppress nematode damage directly, or these could have been disease interactions whereby the fungicides reduced CCN damage indirectly. In either case, this is a positive result indicating potential benefits of applying current-season disease control treatments.

Large, patchy areas of poor crop growth due to CCN damage characterized the trial at Peake. The results from this trial therefore need to be treated with caution. There were no significant effects of treatment on yield (F. pr. = 0.55). In contrast to the Auburn trial, Impact and Tk7a tended to increase the CCN severity, and these treatments also led to reduced yields. This indicates a complex situation where there may be an additional interaction with soil type. The soil at Auburn was a red brown earth of pH (H₂O) 6.5 whereas the soil at Peake was a non-wetting sand of pH (H₂O) 6.7.

Control of take-all and Rhizoctonia in the field, 2001 (Appendix 2)

Three field trials were established: 2 in South Australia and one in Victoria, at sites chosen for a moderate to high risk of severe take-all based on the DNA soil test conducted at C-Qentec / SARDI, Waite campus. Moderately severe take-all was experienced at two of the sites in 2001.

The microbial treatments were formulated according to the method of Yang Hetong, SDAS. These treatments were all applied to the seed as a wettable powder immediately prior to sowing. Biocontrol treatments were compared to chemical fungicides Impact and Jockey (a newly available product in Australia for control of take-all and other diseases). Wheat seed had also been dressed with the usual "seed pickling" fungicides (Raxil, Premis) prior to application of chemical and biological treatments for take-all control.

The full description of the field trials is found in Appendix 2. A notable result was the statistically significant increase in wheat yield at the Goroke (Vic) site following *Trichoderma* treatment (a 5% increase on a wheat yield of 5.8 t/ha), in a field where take-all severity was moderate. The performance of *Trichoderma* at Goroke was equivalent to that of the chemical fungicides Impact and Jockey.

The yield increase at Goroke of 0.3 t/ha at \$180 per tonne of wheat would give a return to the grower of \$55 per ha less the cost of treatment. Treatment costs for Australian conditions are yet to be worked out. Depending on the economics of production, a product that costs of the order of \$10 per ha. would be economic for a farmer to use (this is equivalent to the cost of applying *Penicillium radicum* inoculant to wheat, Australian Seed Inoculants, personal communication).

Despite DNA test predictions, the levels of take-all observed in 2001 were only moderate. At two sites the take-all incidence was 15-20%, which could be expected to decrease grain yield. At the third site there was no take-all. *Rhizoctonia* and *Pythium* damage were also evident at some of the sites.

On Yorke Peninsula, SA, *Rhizoctonia* damage on barley is regarded as a widespread serious problem that cannot easily be controlled. The ability of *Trichoderma* to control *Rhizoctonia* root rot was therefore tested by Wesfarmers and CSIRO at the Foul Bay site, in a small additional trial (restricted to duplicate plots only). It is very noteworthy that *Trichoderma* treatment substantially reduced *Rhizoctonia* severity, from a high rating level of 2.2 to less than 1, which was accompanied by a yield increase of 15%. This work will need to be followed up by replicated trials. Our intention is to try to gain the interest of GRDC and the Australian inoculant industry in further research and development.

Objective 2:

Biocontrol of damping-off of vegetables

Formulation methods for preparing bacterial biocontrol agents was taught by CSIRO staff (R. Warren and P. Harvey) in October/November 2001. Dr Harvey and Ms Warren gave lectures on soil borne pathogen ecology and biocontrol agent ecology. A number of papers have been published from the joint CZU – CSIRO research (see section (v) and Appendix 4 below), in both Chinese and international journals.

Objective 3:

Biocontrol of soil-borne root diseases of cotton (Appendix 3).

Binucleate *Rhizoctonia* strains (BNR1 and BNR2, CSIRO) were tested in field trials in the Xinjiang autonomous region in 2000. BNR2 gave a 13% yield increase, and BNR1 treatment led to a 12% yield increase, while neither substantially decreased seedling disease severity. These biological treatments gave the greatest yield response. In the literature there are numerous reports on the growth and yield promoting properties of BNR, so it is not surprising that yield responses were obtained without effect on disease. Alternatively, the BNR may have controlled later season wilt diseases, which were not assessed in this trial.

In Hebei province in 2000, no yield data were collected due to bollworm damage.

In 2001, large cotton yield increases (20 to 30%,) were obtained following treatment with both chemical and biological fungicides. These increases were, however, not statistically significant. When treatments were ranked, the best performer was the chemical fungicide carbendazol (yield + 30%) followed by a combination of *Streptomyces* X4 (Chinese strain) and BNR2 (+ 27%). Carbendazol increased seedling stand early in the crop growth cycle but BNR2 did not.

The 2001 cotton disease control trial in Henan province showed that several treatments reduced seedling disease and increased seedling fresh weight. Treatment with BNR2 and with *Streptomyces* X4 plus BNR2 gave the best effect. These treatments also led to large yield increases (+ 27% and + 34% respectively, both significant at $P = 0.05$).

A search for a reliable, cheap food base for the production of BNR inoculum revealed that cotton residue and wheat bran were the best of the substrates tested in terms of inoculum biomass. In a pot experiment using the different food bases, treatment with BNR in combination with a bacterium CPF-10 grown on wheat bran produced the best cotton seedling stand, although there were no clear differences between the food bases. A variety of food bases appear to be suitable.

IMPLICATIONS AND PLANS

The ACIAR-funded elements of this project have now come to an end, however there are plans and proposals have been submitted to continue collaboration between CSIRO and CAU, ZU and SDAS.

The ZU group are continuing research on the ecology of soil borne pathogens and biocontrol agents, in cooperation with CSIRO and funded by the National Natural Science Foundation of China.

The CAU team plan to re-establish *Trichoderma* Ta7a as a commercial product in China, and to further test binucleate Rhizoctonia (BNR) for control of cotton diseases in China. This may also lead to commercial production if field results continue to be promising.

Two biocontrol treatments can be considered for use in China and in Australia. One, *Trichoderma koningii* 7a (Tk7a), has been used commercially in China and we now have more data (Auburn 2000 trial; Goroke 2001 trial) on positive field performance on wheat in Australia. This was obtained using a Chinese formulation of the organism. In addition, further field-testing of Tk7a for control of Rhizoctonia bare patch on barley is warranted, based on a preliminary result from the 2001 trial on Yorke Peninsula SA. The next step in Australia is likely to be for CSIRO to seek expressions of interest for commercial development, with a possible co-investment from GRDC. We will continue to work together with our Chinese co-operators towards commercial development in both countries. This would be greatly assisted if the application to AusIndustry were successful.

The second potential biocontrol agent is a binucleate Rhizoctonia (BNR2), which was originally isolated by CSIRO and controlled damping-off of bedding plants and vegetable seedlings. This organism has shown good potential to increase yield in field trials on cotton in China (2000 and 2001). BNR2 is being produced in a small scale production on solid substrate in China (10 kg), for further field trials. This agent could be considered for trials in Australia for the control of Fusarium wilt on cotton, which is a very serious and increasingly important disease for cotton growers in NSW and Qld. A copy of Prof Tang's report could be passed on to the Cotton R&D Corporation for their consideration.

Continued research and development cooperation is in progress and planned as follows:

1. Cooperative research on microbial ecology between ZU and CSIRO, funded by the National Natural Science Foundation, China (ongoing).
2. AusIndustry application for continued cooperation (exchange visits) between CAU, CSIRO and SDAS (submitted 2001, for two to three years' funding). Topic: commercial development of *Trichoderma* and other agents such as binucleate Rhizoctonia for disease control on cotton and wheat in both China and Australia. Progress in this cooperation is also likely to help further progress at ZU on biocontrol for vegetable diseases.
3. Application to AusAID for a workshop on cereal disease management, including possibilities for the deployment of biological disease control treatments (CAU and CSIRO).
4. Cooperation between SDAS and CSIRO: exchange visits on the biological control of plant disease and related topics. (Recently funded by the Chinese Government).

(iv) Problems:

Earlier successful results with biocontrol of vegetable diseases in Zhejiang province could not be repeated. An assessment of the methods used and potential pathogen load in the test soils could assist decisions for future direction of any further R&D work.

(v) Research Reports and Publications (since January 2001):

*** = copy of paper or copy of abstract appended to this report (Appendix 4)**

1. * Chen Xiao-bin, Zhang Bing-xin, Lou Bing-gan and Li Jun-ying. Effect of Plant Growth-Promoting Rhizobacteria on Disease Control of Cucumber Seedlings. *Journal of Zhejiang University (Agricultural and life sciences)* 1999, 25 (6) : 578-582. (previously reported as submitted).
2. * Zhang Bing-xin, Zhang Ping and Chen Xiao-bin (2000) Factors affecting colonization of introduced microorganisms on plant roots. *Chinese Journal of Applied Ecology* 11(6):951-953. (previously reported as submitted).
3. * Zhang Bing-xin and Zhang Ping (2000) Detection of introduced microorganisms in the rhizosphere. *Journal of Zhejiang University (Agric. & Life Sci.)* 26(6):624-628.
4. * Chen Xiao-bin, Zhang Bing-xin, Lou Bing-gan, M.H. Ryder and Xu Zhi-gang. (2000) Studies on identification of plant promoting rhizobacteria of cucumber using biological analysis. *Microbiology* 27(6):403-407. (previously reported as submitted).
5. * Chen Xiao-bin, Zhang Bing-xin. (2000) Advanced Mechanisms of PGPR in the plant rhizosphere. *Journal of Microbiology* 20 (1):38-41.
6. * Chen Xiao-bin, Zhang Bing-xin, Lou Bing-gan and Ryder M. H. (2001) Introduction of the chromogenic gene to plant growth-promoting rhizobacteria of cucumber. *Acta Microbiologica Sinica* 41(3):287-292. (previously reported as submitted).
7. * Lou Bing-gan, Zhang Bing-xin, Hu Liqiang and Maarten Ryder (2001) Resistance to metalaxyl and biological control of *Pythium* spp. *Journal of Plant Protection* 28(1):55-60.
8. * Lou Bing-gan, Zhang Bing-xin and Maarten Ryder (2001) Population dynamics of *Pseudomonas aeruginosa* CR56 in the rhizosphere of cucumber and tomato. *Journal of Zhejiang University (Agricultural and life sciences)* 27 (2): 183-186 .
9. * Harvey P.R., Lou B-G., Warren R.A., Zhang B-X. and Ryder M.H. (2001) Genetic and pathogenic diversity in a population of *Pythium ultimum* from vegetables: implications for disease control. In "Proceedings of the Second Australasian Symposium on Soilborne Diseases" ed I.J. Porter et al., Second Soilborne Diseases Symposium, Victoria, Australia, pp.31-32.
10. * Warren R.A., Yang H. and Ryder M.H. (2001) Survival of formulations of *Trichoderma koningii* isolate 7a and other new biological control agents when stored at different temperatures. In "Proceedings of the Second Australasian Symposium on Soilborne Diseases" ed I.J.Porter et al., Second Soilborne Diseases Symposium, Victoria, Australia, pp.156-156b.
11. * Lou Bing-gan, Zhang Bing-xin, Maarten Ryder and Stephen Barnett. (2002) Identification of bacteria antagonistic to cucumber seedling damping -off. *Journal of Zhejiang University (Agric. & Life Sci.)* 28(1):54-58.
12. Yang Hetong, Tang Wenhua, Chijianguo, Xu Yanke and Wang Jianing (2002) Identification, modes of action and efficacy in controlling ginger bacterial wilt of a biocontrol agent B1031 *Chinese Journal of Biological Control* (in press).
13. Xiao Xing-long, Yang He-tong, Xia Xian-zhi, Xu Yan-ke and Wang Yu-ping (2002) Identification and classification of *Trichoderma* spp. by morphology and soluble protein gel electrophoresis analysis *Shandong Science* (in press) .
14. Yang Hetong, Tang Wenhua, Xu Yanke, Wang Jianing and Yao Wansheng (2002) Delineation of the genus *Trichoderma* and its sub-generic division. *Shandong Science* (in

press).

15. * Lou Bing-gan, Zhang Bing-xin, Maarten Ryder, Rosemary Warren and Paul Harvey. Study on biological control of cucumber seedling damping-off (accepted by Acta Phytomycol Sinica).
16. * Harvey, P.R., Lou, B-G., Warren, R. A, Harch, B. D., Zhang, B-X. and Ryder, M. H. Genetic and pathogenic diversity in a population of *Pythium ultimum* from a South Australian vegetable soil. Mycological Research: accepted subject to modification.

(vi) **Benefits of Research**

Benefits to farmers (China):

Cereal growers: A *Trichoderma* product based on *T. koningii* 7a was produced in China in 1998 and 1999. Approximately 40 to 50 tonnes of inoculum was produced and sold to farmers. The pack size was 100 g and each pack was enough to treat 1 Chinese mu or 667 square metres.

If we assume that the entire product that was sold was also used to treat crops, the total area treated would have been 33,000 ha.

There are new plans at CAU for the commercial deployment of Tk7a in China and discussions have commenced with a company that would be capable of producing commercial inoculum.

Cotton growers: Binucleate *Rhizoctonia* (BNR2) has given good yield increases in the field in 2000 and 2001. Small-scale solid phase fermentation has now been completed, generating about 10 kg of inoculum for use on further field trials. If these are equally successful, the plan is to initiate a commercialisation strategy.

Vegetable growers: subject to further progress in formulation and field testing, vegetable growers could expect improved productivity from the development of biocontrol inoculants for control of damping-off. This is particularly important as chemical fungicides are used routinely, and fungicide resistance among pathogen populations in the field has been documented (including a study that formed part of this project (see 2000 report)).

Potential benefits to Australian farmers:

Benefits for cereal producers would come from the commercial development of *Trichoderma koningii* 7a in Australia. Based on good results from field trials, a commercialisation strategy is being planned. An inoculant would give farmers (including organic farmers) more management options in the control of disease. Access to Chinese formulation expertise has enabled CSIRO to field test a commercial style inoculant in Australia for the first time. This would not have happened without the ACIAR-sponsored cooperation. The *Trichoderma* inoculation is compatible with seed pickling fungicides

There is also a potential future benefit to cotton growers from the use of binucleate *Rhizoctonia*: this depends on further field trials to test for Fusarium control on cotton.

Training benefits: this project and its pilot project predecessor (1994-1995) has delivered training to 4 Chinese scientists for extended periods in Australia and to at least 7 students in China over a period of 7 years. The two-way training benefits have been substantial. Australian scientists have benefited greatly from learning Chinese methods for the formulation of biocontrol treatments.

Benefits for basic science supporting biocontrol research: research on biocontrol mechanisms, pathogen diversity and microbial ecology of biocontrol agents will all benefit the future development and use of biocontrol treatments in China and in Australia.

Projects on *Pythium* diversity and biocontrol agent ecology (CSIRO and ZU) and on mechanisms in biocontrol (CAU and CSIRO) will all have a positive impact. The work should lead to substantial

refinements to the concept of disease management via crop rotation as well as the appropriate use of biocontrol treatments, in an integrated approach. Disease complexes commonly exist in farming systems and there is great potential for the use of soil DNA tests to determine major pathogens which will inform growers about when to use current season controls, when to use crop rotations, and which rotations to use for maximum beneficial effect.

Projects on mechanisms by which biocontrol agents control disease should lead to improved biocontrol performance, either through selection or development of better strains, or through improvements to formulations and delivery methods (CAU and CSIRO).

Higher degrees completed, related to the ACIAR project:

PhD:

Wang Ye, (CAU, 2001). Phloroglucinol-producing bacteria in take-all decline soils in China.

Ma Ping, (CAU, 2001). Ecology of *Verticillium dahliae* and biocontrol of cotton Verticillium wilt.

Masters:

Mr. Zhou Hong-You (CAU, 2001) Occurrence and Biological Control of Fusarium Root Rot in Milk Vetch (*Astragalus adsurgens*). (Including phloroglucinol-producing disease-suppressive bacteria).

3.3 Travel and Meetings since 1/2001

1. **Dr Maarten Ryder** visited CAU(Beijing – Prof. Tang Wenhua and team), Zhejiang University (Hangzhou – Prof. Zhang Bingxin and team) and SDAS (Jinan - Dr Yang Hetong and team) from 4 to 15 June 2001 for the purpose of project management. Approximately three days were spent in each location. Seminars were presented in Jinan and Hangzhou. The main topics for discussion were as follows:
Beijing, 5 to 8 June – field work on cotton disease control; PhD student graduation seminars (Dr Ryder attended the seminar dry runs with discussion sessions for 5 graduating students); potential application to AusAID for a workshop on root disease constraints and their control – this included a visit to AusAID together with Chris Brittenden (ACIAR).
Hangzhou, 9 to 11 June – field trials and the potential to use formulated microbes for inoculation of vegetables; microbial ecology: a talk on marker genes and disease-suppressive soils.
Jinan, 11 to 14 June – Shandong Academy of Sciences. Visit to the SDAS Biotechnology Centre, discussions about future cooperative projects, completion of a biological material exchange agreement. Seminar on disease-suppressive soils (authors Barnett, Ryder and Roget).
2. **Dr Paul Harvey and Ms Rosemary Warren** visited Prof. Zhang's group at ZU (Hangzhou) where they gave lectures. They also visited SDAS (Jinan) and CAU Beijing where they gave lectures on molecular ecology of soil borne plant pathogens to postgraduate students and held discussions about the project and publications. A full report is attached as Appendix 5.

4.4 Conclusions from the project extension

1. Good efficacy of binucleate Rhizoctonia from CSIRO to control cotton disease and to increase yield in trials conducted by CAU in China has been demonstrated. This finding can be followed up in both China and Australia with further field trials and potential for commercial development. *Trichoderma* Tk7a from CSIRO, which was commercialized by CAU for the control of wheat diseases in China in 1998 and 1999, now requires re-registration. Concerning the commercial use of Tk7a in China, Prof. Tang (CAU) is negotiating with Linxian Science & Technology Company, Qinguandao city (located North-east of Beijing). The proposal is for the company to produce small amounts of *Trichoderma* for further field trials. If the company decides to proceed, CAU plans to re-register the organism for commercial use. This process can be facilitated if a grant (applied for) is obtained from AusIndustry. The Beijing-Goettingen Biotech Company may also consider developing *Trichoderma* agents for commercial use.

2. CSIRO Land and Water completed the testing of wheat disease biocontrol using CAU formulation methodology in the 2001 season. Statistically significant yield responses were obtained in a trial in Western Victoria, adding approx 5% yield to a 5.5t/ha crop (worth an extra \$50/ha at \$180 per tonne). Performance was equal to that of chemical fungicides. Results in 2000 at Auburn SA were also promising, with a yield increase of 18% (F. pr. 0.057) which was close to that of Impact chemical fungicide (+24%) in part of the trial (the trial was conducted + and – Temik for control of cereal cyst nematode).
3. Bacterial strains have been formulated for greenhouse testing by Zhejiang University staff and students. Dr Yang Hetong, Shandong Academy of Science (SDAS), developed the formulation and Rosemary Warren and Paul Harvey (CSIRO) taught the methodology during a visit in November 2001.
4. A substantial number of research reports and papers have been published (see Appendix 4 for details).
5. Continued connection between China and Australia:
 - (a) Because of the strong commercial development of biocontrol agents by CAU and SDAS, and the successful use of Chinese formulations in the CSIRO field testing program, there is a good possibility for further commercial development in both countries. Accordingly, a proposal was submitted to AusIndustry for inter-country exchange visits. The success of this proposal will be decided in May 2002. Other Chinese co-operators are also likely to be able to benefit from this continued connection.
 - (b) A proposal to conduct a workshop on crop root disease and their control in NW China is under development by CAU with the assistance of CSIRO, for submission to AusAID. The possible location is Inner Mongolia. If this application can be submitted within the guidelines and the workshop and associated activities can be successfully achieved, this may provide a model that could be adopted in other parts of China.
 - (c) A connection between Zhejiang University and CSIRO continues, on the microbial ecology of biocontrol agents. (Funding: Chinese National Natural Science Foundation (2001 – 2004).

4. Appendices

Research results are contained in Appendices 1, 2, and 3

Appendix 4: Publications

Travel reports (Paul Harvey and Rosemary Warren) are included as Appendix 5.

April 12, 2002

Signed:

Dr Maarten Ryder

CSIRO Land and Water
Project co-ordinator

APPENDIX 1

Research report on biological control of take-all of wheat

Yield data, field trials, 2000

CSIRO Land and Water

Increasing crop production through biological control
of soil-borne root diseases

2000 FIELD TRIALS

REPORT TO ACIAR

Biological control of take-all of wheat in south eastern Australia

**Rosemary Warren and Maarten Ryder
CSIRO Land and Water**

YIELD DATA

AIM

To test the ability of different formulations of *Trichoderma* and bacteria to control take-all on wheat at different locations in southern Australia.

Materials and Methods

Treatments

The treatments were: an untreated control (CK), a chemical fungicide Impact (a.i. 250 ml/L Flutriafol) applied at 400ml/100kg DAP, *Trichoderma pseudokoningii*, isolated from Avon soil (A5MH, CSIRO), *Trichoderma koningii* (T.k. 7a, CSIRO), *Pseudomonas fluorescens*, strain P303 (P303, from Y. Peng, CAAS), *Pseudomonas fluorescens*, strain P32 (P32, from H. Yang, CAU/SDAS) and *Pseudomonas thivervalensis* isolated from Kapunda soil (K208, CSIRO).

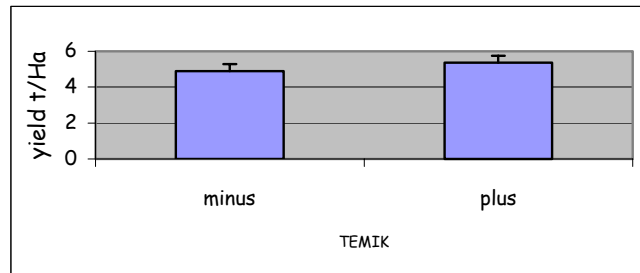
For further details of trial establishment, see 2000 Report to ACIAR.

RESULTS AND DISCUSSION

Trial at Auburn SA, 2000

The soil was infested with cereal cyst nematode, based on the pre-sowing DNA test. The trial was therefore set up as a factorial design, all treatments being applied with and without Temik (chemical nematicide). Grain yield data are shown in Figure 1 and Table 1.

Figure 1. Effect of Temik on yield of wheat at Auburn SA



Although *CCN* severity was low, Temik increased yield by 9.6%, from 4.9 to 5.4 t/ha ($p = 0.017$). Other nematodes, eg *Pratylenchus*, may also have been present and controlled by Temik.

Table 1. Effect of biological and chemical fungicide treatments (minus and plus Temik) on yield of wheat (t/ha) at Auburn SA

TREATMENT	minus TEMIK		plus TEMIK	
	yield	% increase	yield	% increase*
CHECK	4.57	0.0	5.39	17.9
A5MH	4.30	-5.9	5.38	17.7
Tk7A	5.38	17.8	5.40	18.2
IMPACT	5.68	24.3	5.98	30.8
P303	4.61	1.0	5.74	25.5
P32	5.09	11.5	4.39	-3.9
K208	4.65	1.8	5.30	16.0

* % increase relative to check without Temik.

The effect of treatment was close to statistical significance ($P = 0.057$).

In the absence of Temik, three treatments increased yield by more than 10% (Impact fungicide, +24%; *Trichoderma* Tk7a, +18% and *Pseudomonas* P32, +11%).

Interestingly, the disease control data show that there was little take-all damage in this trial and that treatment with Tk7a substantially reduced the incidence and severity of *CCN* (Table 2: note that this is Table 6 from the previous report, reproduced here for convenience).

This is a new observation which would need to be verified, but potentially increases the usefulness of a *Trichoderma* inoculant. The effect of *Trichoderma* on grain yield was equivalent to the effect of Temik (which acted via almost complete control of *CCN*).

Similarly, the effect of Impact (minus Temik) on yield appears to have been mainly via reduction of *CCN* damage.

In the presence of Temik, the only treatments to give additional yield benefits were Impact (additional 13% over Temik alone) and *Pseudomonas* P303 (additional 7.5% over Temik alone). Impact + Temik in combination reduced CCN damage by more than Temik alone. This indicates a potential interaction whereby fungal pathogens may increase CCN damage.

Table 2. Effect of treatment on CCN disease severity and disease incidence at Auburn, 2000

(this is **Table 6** of the 2000 report from CSIRO Land and Water)

	minus Temik		plus Temik		minus Temik		plus Temik	
	severity	PDIDS*	severity	PDIDS*	incidence	PDIDI**	incidence	PDIDI**
Check	0.76	0	0.07	90	39.3	0	7.7	80
A5MH	0.71	7	0.24	69	37.8	4	16.7	58
Tk7a	0.28	63	0.27	64	12.8	67	22.4	43
Impact	0.10	87	0.02	98	7.4	81	1.6	96
P303	0.51	33	0.15	81	35.1	11	13.4	66
P32	0.72	5	0.55	28	38.7	2	29.0	26
K208	0.57	25	0.60	21	25.1	36	30.3	23

*PDIDS - percent decrease in disease severity (over nil Temik check)

**PDIDI - percent decrease in disease incidence (over nil Temik check)

The effect of treatment on grain weight (Table 3) was quite variable. In the absence of Temik, the largest positive effects came from Impact and Tk7a, which also increased yield by the greatest amounts.

Table 3 Effect of treatment on 100-grain weight (g)

Treatment	minus Temik		plus Temik	
		% change		% change
CHECK	3.50	0.0	3.59	0.0
A5MH	3.45	-1.3	3.66	1.8
Tk7A	3.56	1.9	3.51	-2.2
IMPACT	3.62	3.5	3.58	-0.4
P303	3.49	-0.1	3.65	1.5
P32	3.42	-2.3	3.59	-0.1
K208	3.38	-3.3	3.44	-4.1

The effect of treatment on grain protein, as with grain weight, was variable, with no apparent clear pattern (Table 4). Trichoderma Tk7a did not change grain protein, and most other effects of treatment were negative. Impact had a tendency to decrease grain protein in this trial.

Table 4 Effect of treatment on grain protein

Treatment	minus Temik		plus Temik	
		% change		% change
CHECK	12.5	0	12.8	0
A5MH	12.7	1.6	12.5	-2.3
Tk7a	12.5	0	12.8	0
IMPACT	12.2	-2.4	12	-6.3
P303	12.3	-1.6	12.7	-0.8
P32	12.7	1.6	12.2	-4.7
K208	12.4	-0.8	12	-6.3

Trial at Peake, SA, 2000

This trial was severely affected by CCN damage, which was not predicted (and therefore not treated, eg with Temik) because there was too little time to obtain a soil DNA prior to sowing. Results will therefore have to be used with caution.

The yield data are presented in Table 5. The effect of treatment on yield was not significant (F pr. = 0.55). The highest yields came from the check and *Pseudomonas* P32 plots. The lowest yields were recorded from Tk7a, *Pseudomonas* K208 and Impact-treated plots.

Table 5. Effect of treatment on grain yield

Treatment	Grain yield (t/ha)	% change
CHECK	1.43	0.0
A5MH	1.35	-5.9
Tk7A	1.10	-23.1
IMPACT	1.14	-20.6
P303	1.22	-14.8
P32	1.52	5.9
K208	1.09	-23.9

It is noteworthy that the effect of treatment on the severity of CCN in this trial was the reverse of that seen at Auburn (see above). The most severe CCN was seen with Impact (+118%), Tk7a (+48%) and *Pseudomonas* K208 (+37%). There was an inverse relationship between CCN severity and grain yield, indicating CCN as the probable main limitation on yield.

This result indicates a complex situation where Impact and Tk7a interact with CCN, possibly soilborne fungal pathogens diseases, and possibly soil type. The soils at Peake and Auburn are very different: Auburn was a red brown earth of pH (H₂O) 6.5 whereas the soil at Peake was a non-wetting sand of pH (H₂O) 6.7.

Table 6. Effect of treatment on CCN disease severity at Peake

(This is Table 5. from the previous report to ACIAR)

Treatment	Severity	PDIDS*
Check	0.7	0
A5MH	0.8	-8.2
Tk7a	1.1	-47.9
Impact	1.6	-117.8
P303	0.8	-5.5
P32	0.8	-6.8
K208	1.0	-37.0

*PDIDS - percent decrease in disease severity

Table 7. Effect of treatment on grain protein and grain weight

Treatment	protein (%)	% increase	100 grain wt (g)	% change
CHECK	11.5	0	3.43	0.0
A5MH	12.6	10	3.44	0.2
Tk7a	13.1	14	3.41	-0.6
IMPACT	11.5	0	3.52	2.7
P303	12.4	8	3.49	1.9
P32	11.9	3	3.43	0.0
K208	13.3	16	3.36	-2.0

APPENDIX 2

Research report on biological control of take-all of wheat

Field trials, 2001

CSIRO Land and Water

**Increasing crop production through biological control
of soil-borne root diseases**

2001 FIELD TRIALS

REPORT TO ACIAR

Biological control of take-all of wheat in south eastern Australia

**Rosemary Warren and Maarten Ryder
CSIRO Land and Water**

AIM

To test the ability of different formulations of *Trichoderma* and bacteria to control take-all on wheat at different locations in southern Australia.

MATERIALS AND METHODS

Biological control of take-all

Experiments were conducted for the remaining three trials being held over from the 2000 season due to very low predicted take-all levels in 2000. Site selection was based on soil tests carried out by SARDI (Cereal Root Disease Testing Service) for farmer clients. The level of take-all risk at the chosen sites was in the medium to high range (>50 μg of *Ggt* DNA /100g soil). Information for site rainfall, potential cereal root disease levels, soils, soil analysis and sowing are listed in Table 1.

Table 1 Site information and Soil Analyses

Location	COULTA Eyre Peninsula SA	FOUL BAY Yorke Peninsula SA	GOROKE Victoria
<u>INFORMATION</u>			
Location (Latitude Longitude)	-34.383 135.467	-35.000 137.400	-36.717 141.483
Ave annual rainfall mm	520	472.9	495
April-October rainfall (mm)	320	361	346
Previous crop	wheat	wheat	wheat
Take-all risk category	65 μg DNA/g soil	34 μg DNA/g soil	377 μg DNA/g soil
<i>Rhizoctonia</i> risk category	28 μg DNA/g soil	<15 μg DNA/g soil	<15 μg DNA/g soil
CCN risk category	NA	< 1 egg/g soil	< 1 egg/g soil
Plot length, M	30	40	28
Plot width, M	1.4	1.5	1.5
Plot area, M ²	0.004 ha	0.006 ha	0.004 ha
Fertiliser	100 kg ha ⁻¹ DAP	200 kg ha ⁻¹ 32:10:0 and 30 kg ha ⁻¹ DAP	195 kg ha ⁻¹ 32:10:0

Location	COULTA Eyre Peninsula SA	FOUL BAY Yorke Peninsula SA	GOROKE Victoria
Soil type (description)	Grey sandy loam		Grey clay loam
Seed dressing	Premis (1L/tonne)	Premis	Raxil
Potential yield [^]	5.0 t/ha	5.0 t/ha	4.7 t/ha
ANALYSIS	COULTA	FOUL BAY	GOROKE
Physical			
% clay	8.9	NA	54
% silt	2.2	NA	10.8
% fine sand	33.2	NA	17.8
% coarse sand	47.6	NA	13.4
% total	98	NA	96
Chemical			
pH H ₂ O(1:5 susp)	5.5?	8.3	7.3
pH CaCl ₂ (1:5 susp)	4.8?	7.5	6.7
EC dS/m	0.12	0.18	0.17
Cl (ppm)	NA	NA	NA
% Org C	1.1	3.05	2.4
% Total N	0.09	NA	0.23
NH ₄ -N (ppm)	1.1	3.0	4.2
NO ₃ -N (ppm)	12.7	11.0	41.2
HCO ₃ -ext P (ppm)	49	49	29
HCO ₃ -ext K (ppm)	570	109	830
C.E.C (NH ₄)	8.1	NA	35.5
% CaCO ₃	6.0	NA	NA

[^] April- October rainfall (mm) - 110) x 0.02

Treatments

Chemical controls

Commercial chemical treatments, used to control take-all in wheat, of Impact, (a.i. 250 ml/L Flutriafol) applied at 400ml/100kg to DAP and Jockey Flexi, (a.i. 167 g/L Fluquinconazole) applied to seed at between 0.4L and 0.45L/100 kg were trialed with formulations of fungi and bacteria.

Bacteria and fungi

The treatments were 3 strains of *Trichoderma koningii* isolate 7A (Tk 7A), isolate 7C (Tk 7C) and isolate AST1 (Tk AST1) isolated by CSIRO in Western Australia (Simon and Sivasithamparam 1988) and *Pseudomonas thivervalensis* (K208) isolated by Y.Alami (Ross *et al*/2000) from soil collected at Kapunda SA. An untreated seed control (CHECK) was also included.

Wheat variety and sowing rates

100 kg ha⁻¹ *Triticum aestivum* var. Krichoff was sown at Coultas, 90 kg ha⁻¹ *T. aestivum* var. H45 was sown at Foul Bay and 100 kg ha⁻¹ *T. aestivum* var. Babbler at GoroKe.

Fertiliser

For fertiliser treatments and rates refer to Table 1.

Inoculum production

Formulations of the bacteria were prepared by subculturing *Pseudomonas thivervalensis* from freezer stocks and streaking for pure culture on King's medium B. Plates were incubated for 2-3 days at 25°C. Plates were re-streaked and incubated a further 2-3 days at 25°C. The bacteria were transferred to 200 ml lots of Kings B broth, (total culture volume = 1200ml) and shake-cultured at 25°C for 2-3 days until the cell density reached 6.7×10^9 cfu ml⁻¹ (A_{550} of 1 ~ 10^9 cfu/ml). Counts were also estimated under a microscope using a haemocytometer.

Bacteria were centrifuged 6090xg at 4°C for 10 minutes. The pellets of bacteria were collected and mixed with 7.5gm Xanthan Gum (Sigma). Cells were refrigerated overnight and mixed with 500gm commercial grade attapulgate, which had been ground and sieved through a 60-mesh sieve. The formulation was dried under a biosafety hood, then re-ground and final counts (cfu/gm) were made using the dilution plate method.

Formulations of the isolates of *Trichoderma* were prepared by inoculating plates of PDA + 20ppm triadimefon with 100µl suspensions of conidia of *Trichoderma koningii* isolates 7A, 7C and AST1 growing on PDA amended with 20ppm triadimefon which had been incubated on the laboratory bench in natural light. The plates of the *Trichoderma* conidia suspensions were further incubated under 12hr periods of a black light 40 watt fluorescent lamp (NEC) followed by 12hr periods of dark for 7 days.

Ten ml of sterile distilled water was added to each plate and a loop was used to loosen the conidia. They were transferred into sterile 500ml centrifuge bottles and the plates re-washed 4 x each with 5ml sterile distilled water. Suspensions were spun 13,700xg for 10 minutes in a Sorvall RC2B centrifuge fitted with GS3 head. The supernatant was poured off and the cells were collected. Counts were estimated using haemocytometer. There was an average number of 1×10^7 /ml for each strain.

The separate suspensions of conidia were mixed thoroughly with dried and ground autoclaved rye grass seed amended with cornmeal, FeSO₄ and streptomycin to stop bacterial growth. It was sieved through a 100-mesh sieve and propagule counts (cfu/gm) were estimated using dilution plate method. The final formulated products contained 2.0×10^8 cfu/gm for isolate Tk7A, 2.0×10^7 cfu/gm for isolate Tk7C and 7.5×10^7 cfu/gm for isolate TkAST1.

Seed inoculation

Seed were treated immediately prior to sowing by placing untreated seed in a bucket and adding water at 8% of the seed dry weight. The formulation was placed on to the wetted seed immediately and the mixture stirred well. Seed were then air-dried approximately 60 minutes and sown using a cone seeder.

Formulations were applied at 1% w/w. Bacteria were applied at an average of 3.2×10^5 cfu per seed. *Trichoderma* isolates were applied at an average of 1.3×10^4 cfu per seed.

Additionally, at Foul Bay, 2 replicate treatments of barley, *Hordeum vulgare* var. Sloop, treated with Baytan and inoculated with *Trichoderma koningii* isolate 7A were sown with 2 replicate treatments of an uninoculated control. Fertiliser treatment was the same as for wheat.

Counts on seed were made the day after sowing.

Experimental design

The experimental design was a one factor (7 levels) RCBD and was replicated 4 times so that the total number of plots was 28. At Coult and Foul Bay the plots were located adjacent to each other. At Goroke block 3 was located above block 1 and block 4 above block 2.

Statistical analysis

Data were analysed by ANCOVA in conjunction with nearest neighbour analysis. Nearest neighbour analysis takes into account patchiness in disease in the field and utilises the data for all plots adjoining a particular plot in the analytical process. Yield data for the Goroke trial were analysed by multiple comparisons using linear contrasts. Data were analysed using Genstat 5 release 4.2. Dr Ray Correll, CSIRO Mathematical and Information Sciences, assisted with the nearest neighbour analysis.

Sampling and Disease assessment

Between 50 and 60 plant samples were taken at random from each plot 12 weeks after sowing for disease and shoot weight assessment. Shoots were oven-dried and weighed, the numbers of tillers were counted and roots were rated for disease damage.

Take-all

Take-all root disease severity was assessed by (a) measuring the proportion of seminal roots with take-all lesions, disease severity and (b) percent incidence, the proportion of plants affected by take-all. This takes into account the plants with different numbers of seminal roots.

Rhizoctonia

Rhizoctonia disease damage was assessed (a) on a 0-5 scale (McDonald and Rovira, 1985)

where 0 = no disease, 1 = < 25% of primary roots with infected root tips/cortical rot, 2 = 25 - 50 % of primary roots with infected root tips/cortical rot, 3 = 50 - 75% of primary roots with cortical rot and truncation, 4 = > 75% of primary roots with cortical rot and severe truncation and 5 = primary roots severely

truncated, top stunted and moribund and by (b) percent incidence, the proportion of plants affected by *Rhizoctonia* disease.

RESULTS

Effect of treatment on shoot dry weight (mg), 12 weeks after sowing

There was no significant ($p=0.05$) effect of treatment on shoot dry weight per plant at Coultla, Foul Bay or Goroke (Table 2). At Coultla application of all the treatments increased shoot dry weight per plant by between 18 and 55%. At Foul Bay, greater shoot dry weights were achieved with the applications of Impact, Jockey, *Trichoderma* isolates 7A and 7C and with K208. The application of Jockey, all the *Trichoderma* isolates and K208 increased shoot dry weight by up to 27.4% at Goroke.

Table 2. Average shoot dry weights per plant 12 weeks after sowing (mg/plant)

treatment	COULTA (% change*)	FOUL BAY (% change*)	GOROKE (% change*)
Check	1244 (0)	326 (0)	1041 (0)
Impact	1473 (+ 18.4)	377 (+ 15.6)	975 (- 6.3)
Jockey	1531 (+ 23.1)	372 (+ 14.1)	1178 (+ 13.2)
Tk7A	1586 (+ 27.5)	337 (+ 3.4)	1061 (+ 1.9)
Tk7C	1479 (+ 18.9)	390 (+ 19.6)	1156 (+ 11.1)
TkAST1	1497 (+ 20.3)	282 (- 13.5)	1098 (+ 5.5)
K208	1938 (+ 55.8)	331 (+ 1.5)	1326 (+ 27.4)

* percent increase (+) or decrease (-) over check

Effect of treatment on numbers of tillers, 12 weeks after sowing

There was no significant ($p=0.05$) effect of treatment on the numbers of tillers per plant at Coultla, Foul Bay or Goroke (Table 3). However at the $p=0.10$ level, there was a significant effect of treatment on numbers of tillers per plant at Foul Bay ($l_{sd} = 0.160$). Compared to the untreated check the numbers of tillers per plant was significantly increased by applications of Tk7C and Impact. At Coultla and Goroke the greatest numbers of tillers was achieved with applications of K208 (44.1% increase) and Tk7C (21.2% increase) respectively.

Table 3. Average numbers of tillers per plant 12 weeks after sowing

treatment	COULTA (% change*)	FOUL BAY (% change*)	GOROKE (% change*)
Check	3.1 (0)	1.12 (0)	3.11 (0)
Impact	3.5 (+10.9)	1.36 (+27.3)	3.06 (-1.6)
Jockey	3.5 (+11.5)	1.24 (+9.1)	3.54 (+13.8)
Tk7A	4.0 (+26.8)	1.17 (+9.1)	3.28 (+5.5)
Tk7C	3.8 (+19.8)	1.31 (+18.2)	3.77 (+21.2)
TkAST1	3.7 (+18.2)	1.20 (+9.1)	3.74 (+20.3)
K208	4.51 (+44.1)	1.20 (+9.1)	3.61 (+16.1)

* percent increase (+) or decrease (-) over check

Effect of treatment on take-all disease, severity

There was no significant effect of treatment on take-all disease severity at Foul Bay or Goroke (Table 4). The numbers of seminal roots with take-all lesions was reduced by all the treatments at Foul Bay and by applications of the chemical controls, *Trichoderma* isolates 7A and AST1 and by K208 at Goroke. Disease severity was increased by the application of *Trichoderma* isolate 7C at Goroke but this was not significant. There was no take-all disease at Coultla.

Table 4. The percent of seminal roots per plant with take-all lesions 12 weeks after sowing.

treatment	COULTA (% change*)	FOUL BAY (% change*)	GOROKE (% change*)
Check	No disease	8.4 (0)	5.9 (0)
Impact	No disease	4.1 (- 51.4)	2.6 (- 55.9)
Jockey	No disease	6.7 (- 19.9)	4.5 (-23.7)
Tk7A	No disease	4.4 (- 47.3)	3.6 (- 39.0)
Tk7C	No disease	6.8 (-19.2)	10.6 (+ 79.7)
TkAST1	No disease	4.7 (- 43.6)	2.3 (- 61.0)
K208	No disease	7.0 (-16.8)	5.2 (- 11.9)

* percent increase (+) or decrease (-) over check

Effect of treatment on take-all disease, incidence

There was no significant effect of treatment on the proportion of plants with take-all lesions (Table 5). The incidence of take-all disease was reduced at Foul Bay by all treatments and by the chemical controls, *Trichoderma* isolates 7A and AST1 and K208 at Goroke. The proportion of plants affected by take-all was increased by the application of *Trichoderma* isolate 7C at Goroke but this was not significant. There was no take-all disease at Coultla.

Table 5. The proportion of plants (%) with take-all lesions, 12 weeks after sowing.

treatment	COULTA (% change*)	FOUL BAY (% change*)	GOROKE (% change*)
Check	No disease	18.1 (0)	16.6 (0)
Impact	No disease	13.2 (- 27.1)	9.5 (- 42.8)
Jockey	No disease	17.3 (- 4.4)	14.4 (- 15.1)
Tk7A	No disease	15.7 (- 13.3)	11.7 (- 29.5)
Tk7C	No disease	16.6 (- 8.3)	22.1 (+ 33.1)
TkAST1	No disease	13.6 (- 24.9)	9.5 (- 42.8)
K208	No disease	14.5 (-19.9)	10.0 (-39.8)

* percent increase (+) or decrease (-) over check

Effect of treatment on *Rhizoctonia* disease, severity

There was no significant ($p=0.05$) effect of treatment on *Rhizoctonia* disease severity at Coultla, Foul Bay or Goroke (Table 6). At Foul Bay, applications of Impact, Tk7A and K208 reduced *Rhizoctonia* disease severity by 47.9, 40.9 and 36.6% respectively while applications of Jockey and Tk7C increased it. Disease severity with the application of AST1 was the same as the untreated control. At Coultla, applications of all treatments increased disease severity while at Goroke disease severity with Tk7A was the same as the check but was increased with all other treatments.

It was notable that at all trial sites the application of Jockey increased *Rhizoctonia* disease severity, by between 29.8 and 67.6%.

Table 6. *Rhizoctonia* disease severity (0-5 scale) at 12 weeks after sowing

treatment	COULTA (% change*)	FOUL BAY (% change*)	GOROKE (% change*)
Check	1.5 (0)	0.7 (0)	1.8 (0)
Impact	2.1 (+ 38.8)	0.4 (- 47.9)	2.1 (+ 16.6)
Jockey	2.4 (+ 54.6)	1.2 (+ 67.6)	2.4 (+ 29.8)
Tk7A	1.8 (+ 15.1)	0.4 (- 40.9)	1.8 (- 0.6)
Tk7C	1.9 (+ 26.3)	0.8 (+ 11.3)	2.2 (+ 23.8)
TkAST1	1.9 (+ 25.7)	0.7 (+ 2.8)	2.2 (+ 19.9)
K208	1.7 (+ 13.8)	0.5 (- 36.6)	2.3 (+ 24.9)

* percent increase (+) or decrease (-) over check

Effect of treatment on *Rhizoctonia* disease, incidence

There was no significant ($p=0.05$) effect of treatment on the proportion of plants with *Rhizoctonia* disease 12 weeks after sowing at Coultla, Foul Bay or Goroke (Table 7). The numbers of plants infected with *Rhizoctonia* disease varied from an average 44% at Foul Bay to high levels (80-100%) at Coultla and Goroke. At Foul Bay, where *Rhizoctonia* disease incidence was the lowest, a reduction in the proportion of plants with disease was achieved with applications of Impact, Jockey, *Trichoderma* isolates 7A and AST1 and with K208. At Coultla and Goroke, changes compared to the untreated check were slight and ranged from an increase of 10% (ca 10 out of 100 plants) at Coultla to a 4.8% increase (ca 5 out of 100 plants) at Goroke.

Table 7. The proportion of plants with *Rhizoctonia* disease 12 weeks after sowing

treatment	COULTA (% change*)	FOUL BAY (% change*)	GOROKE (% change*)
Check	82.2 (0)	50.5 (0)	94.6 (0)
Impact	87.8 (+ 6.7)	27.8 (- 45.0)	96.8 (+ 2.3)
Jockey	88.1 (+ 7.2)	43.4 (- 14.1)	98.8 (+ 4.4)
Tk7A	79.2 (- 3.6)	42.2 (- 16.4)	99.2 (+ 4.8)
Tk7C	87.9 (+ 6.9)	60.1 (+ 19.0)	93.2 (- 1.6)
TkAST1	90.6 (+ 10.2)	43.6 (- 13.7)	98.5 (+ 4.1)
K208	80.5 (- 2.1)	44.5 (- 11.9)	97.0 (+ 2.4)

* percent increase (+) or decrease (-) over check

Effect of treatment on *Pythium* root rot

At the Foul Bay site, there was a high incidence of *Pythium* on wheat (50% incidence). There was no effect of treatment (data not shown).

Effect of treatment on yields

A complex statistical analysis revealed an effect of treatment at Goroke. Application of both the chemical treatments, *Trichoderma* isolates 7A, 7C and AST1 as well as *Pseudomonas thivervalensis* (K208) significantly increased the yields above the nil control. There was no significant ($p=0.05$) effect of treatment on grain yields at Coultla and Foul Bay (Table 8). At Coultla application of Jockey produced the best yields. Yields at Foul Bay were slightly reduced by all the treatments although this was not significant

Table 8. Grain yields (t/ha)

treatment	COULTA (% change*)	FOUL BAY (% change*)	GOROKE (% change*)
Check	4.1 (0)	2.3 (0)	5.8 (0)
Impact	3.9 (+ 4.9)	2.1 (- 10.3)	6.0 (+ 4.2)
Jockey	4.2 (+ 2.7)	2.1 (- 9.1)	6.1 (+ 5.3)
Tk7A	4.1 (-0.5)	2.1 (- 8.1)	6.1 (+ 4.8)
Tk7C	4.0 (- 1.5)	2.1 (- 9.9)	6.1 (+ 5.2)
TkAST1	4.0 (- 2.0)	2.0 (- 12.5)	6.1 (+ 5.3)
K208	4.1 (+ 1.5)	2.0 (- 11.1)	6.0 (+ 4.3)
<i>lsd</i>	<i>n.s.</i>	<i>n.s.</i>	0.206

* percent increase (+) or decrease (-) over check

Effect of treatment on 100 grain weights

There was a significant ($p=0.05$) effect of treatment on 100 grain weights at Foul Bay, $lsd = 0.131$ (Table 9). Compared to the untreated check grain weight was significantly increase by applications of Impact, Jockey, Tk7A and K208. At Coultla and Goroke changes compared to the untreated check were slight. The

greatest increases were achieved with applications of K208 at Couлта (increased 1.88%) and with Jockey at Goroke (increased 10.35%).

Table 9. 100 Grain weights (grams)

treatment	COULTA (% change*)	FOUL BAY (% change*)	GOROKE (% change*)
Check	3.7 (0)	2.9 (0)	2.9 (0)
Impact	3.8 (+ 1.69)	3.2 (+ 8.2)	3.2 (+ 8.2)
Jockey	3.8 (+ 0.81)	3.2 (+ 10.4)	3.2 (+ 10.4)
Tk7A	3.8 (+ 0.86)	3.1 (+ 6.1)	3.1 (+ 6.1)
Tk7C	3.7 (- 0.35)	3.0 (+ 2.4)	3.0 (+ 2.4)
TkAST1	3.8 (+ 1.02)	3.0 (+ 4.0)	3.0 (+ 4.0)
K208	3.8 (+ 1.88)	3.1 (+ 4.7)	3.1 (+ 4.7)

* percent increase (+) or decrease (-) over check

Effect of treatment on grain protein (%)

Grain samples were bulked for protein analysis and the results represent a single sample. Therefore no statistical analysis was performed.

Impact showed a tendency to decrease grain protein.

treatment	COULTA (% change*)	FOUL BAY (% change*)	GOROKE (% change*)
Check	10.5 (0)	11.0 (0)	12.1 (0)
Impact	9.9 (- 5.4)	11.2 (+ 1.6)	11.6 (- 4.7)
Jockey	10.5 (0)	11.2 (+ 1.6)	12.4 (+ 1.9)
Tk7A	10.1 (- 3.3)	11.2 (+ 1.6)	12.4 (+ 2.3)
Tk7C	10 (- 4.3)	11.2 (+ 2.1)	12.3 (+ 0.9)
TkAST1	10.2 (- 2.7)	11.4 (+ 3.6)	12.3 (+ 0.9)
K208	10.4 (- 0.5)	11.5 (+ 4.1)	12.3 (+ 0.9)

* percent increase (+) or decrease (-) over check

Effect of treatment on barley plant growth, disease control and yields (Foul Bay, Yorke Peninsula, SA).

Results represent 2 replicate samples for each treatment and so a complete statistical analysis could not be performed. Results are averages of the two representative treatments.

Plant growth

The application of *Trichoderma koningii*, isolate 7A improved average shoot dry weight and numbers of tillers per plant (at 12 weeks after sowing) by 27% and 3% respectively (Table 11).

Take-all control

There was a 71% reduction in the proportion of plants affected by take-all disease where *Trichoderma* isolate 7A was applied to barley seed prior to sowing. Additionally there was a 2% reduction in the proportion of seminal roots with take-all lesions (Table 11).

Rhizoctonia control

Rhizoctonia root rot severity (rating) was reduced from 2.15 to 0.89 where Tk7A was applied and the numbers of plants with disease symptoms was reduced by 61% (Table 11).

Yields

Where *Trichoderma koningii*, isolate 7A was applied to barley seed prior to sowing grain average yield at harvest was improved by more than 10% (Table 11).

Table 11. Effect of treatments on barley plant growth, disease severity, yields and grain weights

Treatment	untreated	plus Tk7A	% change*
Shoot dry weight(mg/plant)	388.4	531.4	+ 36.8
Numbers tillers/plant	2.6	2.7	+ 3.5
Take-all incidence ¹ (%)	10.7	6.3	-41.7
Take-all severity ³	0.2	0.2	-2.0
Rhizoctonia incidence ² (%)	91.8	57.0	-38.0
Rhizoctonia severity ⁴	2.2	0.9	-58.5
Pythium incidence(%)	52.4	43.9	-16.2
yield (t/ha)	1.3	1.6	+ 15.4
Grain weight	2.7	2.7	0
Grain protein	11.2	11.9	+ 6.2

1. The proportion of seminal roots with take-all lesions 2. The proportion of plants affected by take-all disease 3. Proportion of plants affected by *Rhizoctonia* disease. 4. *Rhizoctonia* disease damage (0-5).

* percent increase (+) or decrease (-) over check

DISCUSSION

Plant growth of wheat

Treatments did not significantly improve shoot dry weight (Table 1) although there was a trend towards increased shoot dry weights by treatment with Jockey, *Trichoderma* isolates 7C and AST1 as well as *Pseudomonas* K208.

Treatments significantly affected the numbers of tillers per plant at Foul Bay, $p = 0.1$ (Table 2). Numbers of tillers per plant were significantly improved with applications of Tk7C and Impact. At Coultla and Goroke the greatest numbers of tillers was achieved with applications of K208 and Tk7C respectively.

Biocontrol of take-all of wheat

Levels of take-all (both incidence and severity) tended to be low despite predictions from the soil DNA test. There was no significant effect of treatment on take-all disease at Foul Bay or Goroke (Tables 3 & 4). Take-all disease severity and incidence was reduced (n.s.) by all the treatments at Foul Bay and by all the treatments except *Trichoderma* isolate 7C at Goroke. There was no take-all disease at Coultla.

Biocontrol of *Rhizoctonia* of wheat

There was no significant effect of treatment on *Rhizoctonia* disease severity or incidence at Coultla, Foul Bay or Goroke (Tables 5 & 6). Application of Impact, Tk7A and K208 at Foul Bay reduced *Rhizoctonia* disease severity while treatments Impact, Jockey, Tk7C, AST1 and K208 increased it at Goroke. Application of *Trichoderma* isolate 7A increased *Rhizoctonia* disease severity at Coultla but was the same as the nil control at Goroke. Where *Rhizoctonia* disease incidence was the lowest, a reduction in the proportion of plants with disease was achieved with applications of Impact, Jockey, *Trichoderma* isolates 7A and AST1 and with K208. Disease incidence and severity was high at Coultla and Goroke and may have concealed any treatment effects. Although not significant, chemical applications at Coultla and Goroke increased *Rhizoctonia* disease severity.

Yields and grain analysis of wheat

There was a significant effect of the treatments on yield at Goroke (Table 7). All the treatments significantly increased the yields above the nil control. Grain weights were significantly increased by applications of Impact, Jockey, Tk7A and K208 at Foul Bay (Table 8). Grain protein results for each site represents a single sample from bulked same treatments and so a statistical analysis was not performed.

Plant growth, disease control and yields of Barley

Although no valid statistical comparison could be made, the application of *Trichoderma koningii*, isolate 7A improved shoot dry weight and numbers of tillers per plant. Additionally the proportion of plants affected by take-all disease as well as the number of seminal roots with take-all lesions was reduced where *Trichoderma* isolate 7A was applied to barley seed prior to sowing. *Rhizoctonia* root rot severity and the numbers of plants with disease symptoms was reduced where Tk7A was applied. This is important as *Rhizoctonia* disease is generally a more dominant problem in barley than in wheat and there are currently few control options.

Average grain yield at harvest was improved by 15% where *Trichoderma koningii*, isolate 7A was applied.

CONCLUSIONS

Treatments Tk7C and Impact significantly improved the numbers of tillers per plant in a calcareous sand at Foul Bay.

At Foul Bay where *Rhizoctonia* disease incidence was moderate, a reduction in the proportion of plants with disease was achieved with applications of Impact, Jockey, *Trichoderma* isolates 7A and AST1 and with *Pseudomonas* K208.

Although not significant, chemical applications at Coult and Goroke increased *Rhizoctonia* disease severity.

Significant increases in yield at Goroke and grain weight at Foul Bay were achieved.

Rhizoctonia root rot is an economically important disease of barley particularly on Eyre Peninsula, a major cereal growing area of Southern Australia. The promising result with barley at Foul Bay needs to be verified with a statistically designed field trial.

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APPENDIX 3

Research report on biological control of cotton diseases

Field and glasshouse trials, 2000 and 2001

China Agricultural University

RESEARCH REPORT TO ACIAR ON THE EXTENDED PROGRAM ACIAR PN9680

EVALUATION OF EFFECT ON CONTROL OF SEEDLING DISEASE OF COTTON BY BINUCLEATE RHIZOCTONIA STRAINS

Based on earlier research results in the project “ACIAR PN9680”, the CAU group became very interested in binucleate Rhizoctonia (BNR) for the control of cotton seedling diseases. This work became part of extended project. The CAU group received BNR1 and BNR2 strains from Dr. Maarten Ryder in April 2000, then started this research. The CAU staff involved in this research included Tang Wenhua, Wang Ye, Zhang Liqun and Yang Hetong. Since then, the two strains have been tested in the laboratory and greenhouse, and in field plots for two years in three provinces in China. Ms Yan Xioxue coordinated experiments conducted in the Xinjiang region, Dr. Li Hong-lian coordinated experiments in Henan and Dr. Ma Ping was responsible for experiments conducted in Hebei province. The purpose of the experiments in first year was to compare binucleate Rhizoctonia with previously selected biocontrol agents in field tests for disease control. The purpose of experiments conducted in second year (2001) was to reconfirm the effect of BNR strains on increasing yield of cotton. In the first year, data collected from Xinjiang showed that treatment with binucleate Rhizoctonia increased yields compared with the check and other treatments. In the first year, we did not collect yield data at Baoding. In the second year, we did not collect yield data in Xinjiang, due to plants being damaged by extremely cool conditions. The remaining information was collected from experiments done in each of the two years.

Field experiment in Shihezi, Xinjiang in 2000:

Materials and methods:

1. Background of experimental field: It is located at No.1, 2nd Dao field, which belongs to the Agricultural Technology Station of the 121st Military Group, Xinjiang. Area of the field is 3.3 ha. The crop grown in this field in the previous growing season was cotton. The soil is loamy.
2. Treatments and design of the field experiment:
There were 7 treatments with 3 replicates designed in randomized complete block. Total number of the plots was 21 with 11 m² for each plot. The treatments included: BM (*Bacillus* mixture); CPf-10 (*Pseudomonas*); PBM (*Pseudomonas* – *Bacillus* mixture; BNR 1; BNR 2; Baytan and Check.
3. Method of seed treatment:
The dosage of biocontrol agents used for seed treatment was 1.5 % (w/w). First, the seeds were treated with 1 % 3911 (an insecticide) including 60% a.i., then the seeds were treated with the biocontrol agents and air dried.
4. BNR 1 and BNR 2 agents were prepared by cutting the mycelium into pieces with a grinder in solution then soaked in CaCl₂.
5. Investigation:
Investigation of the disease index was made 10 days after seedling emergence. Assessment of yield was made on August 24, 2000.
Assessment of disease
0: healthy seedling
1: small lesions or color change at the seedling base or on the roots.
2: lesions extended to 1/3 to 1/2 of the base of stem or roots.
3: lesions extended to cover the base of the stem.
4: seed rotted or seedling wilted or dead.

Disease incidence (%) = Number of diseased seedlings / Total number of seedlings assessed

Disease index = (assessment number x responsive number of seedlings) / 4 x disease index

Results

Table 1 shows that the effectiveness of biocontrol agents for control of seedling diseases was lower than for the chemical control, Baytan, according to data from investigations made on May 10 and May 20. Compared to the non-treated control, BNR2 expressed a certain level of effectiveness in controlling disease, but other biocontrol agents did not.

Table 1. Effectiveness of biocontrol agents for control of seedling disease of cotton

Treatment	Date of emergence	Date of investigation, May		Date of investigation, May	
		10	10	20	20
		Disease incidence	Disease index	Disease incidence	Disease index
BM	5/6	70.7	30.5	63.3	25.8
CPF-10	5/6	55.3	19.7	50.0	20.8
PBM	5/6	61.3	22.7	43.3	15.8
BNR1	5/6	64.7	26.8	53.3	17.5
BNR2	5/6	34.0	12.7	36.7	13.3
Baytan	5/6	19.3	7.2	40.0	11.7
Check	5/6	44.7	18.5	43.3	14.2

2. At the end of the growing season, investigations were made on the numbers of cotton plants per hectare, numbers of bolls per plant, weight per boll and yield per hectare. The data are listed in Table 2.

We can list the following results from Table 2:

- A. The number of plants per ha differed between treatments, with more plants occurring where seed was treated with BNR2, Baytan and CPF-10 compared to the Check.
- B. In response to the treatments, it seems that both biological and chemical agents increased numbers of bolls per plant. BNR 1 was most effective, then BM, PBM and Baytan. BNR 2 took fifth place and CPF-10 was the sixth.
- C. There was no difference between treatments in weight per boll.
- D. It is very interesting to note the yield data. BNR2 and BNR1 performed very well in the field experiment, although fungicide Baytan was effective in controlling seedling disease and increasing yield as well.

Table 2. Effect of biocontrol agents on the yield of cotton

Treatment	Number of plants / ha	Number of bolls / plant	Weight per boll (g)	Yield (kg/ha)	Increase in yield	
					Per hectare (kg)	percentage
BM	160,000	5.93	4.7	4,460	171	4.0
CPF-10	169,995	5.87	4.7	4,691	402	9.1
PBM	160,000	5.93	4.7	4,460	171	4.0
BNR1	160,000	6.37	4.7	4,791	502	11.7
BNR2	175,000	5.90	4.7	4,853	564	13.2
Baytan	169,995	5.93	4.7	4,739	450	10.5
Check	165,000	5.53	4.7	4,289	0	0

Discussion

- A. According to reports (1, 2), prevalent pathogens that cause seedling diseases on cotton in Xinjiang are *Rhizoctonia solani* AG-4 and *Fusarium* spp. The severity of seedling diseases ranged from low to medium in the experimental field.
- B. The effectiveness of biological agents and the chemical fungicide on control of seedling diseases of cotton in field was well recorded. Baytan and BNR2 reduced disease incidence and index compared to the non-treated check. BM, CPf-10, PBM and BNR1 increased severity of the diseases.
- C. At the end of growing session, the number of plants per hectare differed between treatments, with BNR2 > Baytan, CPf-10 > Check > BM, PBM, BNR1. The number of bolls per plant was different in response to treatment, with BNR1 > BM, PBM, Baytan > BNR2 > CPf-10 > Check. Comparing the data in Tables 1 and 2, it seems that the tendency between disease severity and the number of plants per plot was a positive correlation except for CPf-10 treatment, but the difference was not significant.
- D. The number of bolls per plant was quite different in response to treatment. This may be the main factor in increasing yield.

Conclusion

Based on the results obtained from field experiment in Xinjiang, BNR2 and BNR1 are promising biocontrol agents and worth to continue the experiment on the evaluation of effectiveness in controlling the seedling diseases of cotton.

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Field experiment on biocontrol of seedling diseases of cotton in Baoding, Hebei Province, 2000.

Materials and methods

Basically, these were the same as for the description of the experiment conducted in Xinjiang. There were just several differences, which should be pointed out, as follows:

1. 70% Streptomycin was used as the chemical control instead of Baytan. The dosage of Streptomycin used to treat seeds was 3.75g / kg seed weight.
2. Cultivar of cotton used in this experiment was Ji-Mian No.1
3. Planting date was postponed due to waiting for BNR agents.
4. No yield data were collected due to damage from boll worm.
5. Soil in the experimental plot was a loamy sand. Cotton was cultivated in this field for the previous 3 years.
6. The design of the experiment was the same as in Xinjiang, with the size of one plot being 33m². Total number of plots was 21.
7. Statistical analysis was made using Duncan's multiple range test.

Results

1. There was no significant difference between treatments except for the data obtained from treatment with Streptomycin and CPf-10 in the first observation (Table 3).
2. It seems that CPf-10 may reduce the severity of seedling disease.

Discussion

1. The planting date of cotton at Baoding was earlier than Xinjiang. This experiment was planted on March 14, which was later than normal.
2. According to the experience from Xinjiang, it is hard to make conclusions from this experiment without yield data.

Table 3. Effect of biocontrol agents and Streptomycin in controlling seedling diseases of cotton in Baoding, Hebei province.

Treatment No.	Treatment	First investigation		Second investigation		Third investigation	
		Disease incidence	Disease index	Disease incidence	Disease index	Disease incidence	Disease index
4	CK	86.8	41.97a	76.5	35.07ab	99.4	55.47ab
7	BNR1	90.6	40.40ab	81.3	39.57a	100.0	56.57ab
3	BM	82.6	37.37abc	79.2	37.63ab	99.5	57.03ab
5	PBM	88.6	36.93abc	81.5	39.50a	100.0	60.17a
1	BNR2	86.9	35.73abc	73.7	35.67ab	100.0	57.97ab
2	CPf-10	81.1	35.17bc	71.5	32.00ab	100.0	56.57ab
6	70% Streptomycin	76.4	31.87c	70.1	30.43b	99.3	51.80b
LSD (0.05)		6.74		8.89		7.86	

Field experiments on biocontrol of seedling diseases by Binucleate Rhizoctonia in 2001

A. Experiment conducted at Baoding, Hebei province

Materials and Methods

1. *Streptomyces* X4 and BNR2 as biocontrol agents, which were offered by Biocontrol Laboratory, CAU
2. Chemical Check: 50% carbendazol, which was produced by Jiangsu Xinxin Pesticide Factory
3. Target diseases: Seedling diseases of cotton, caused mainly by *Rhizoctonia solani*. *Pythium* and *Fusarium* were also present.
4. Cultivar of cotton: Xinmian 33
5. Method of treatment: see Table 1.
6. Design of experiment: Three replicates for each treatment, random block design, area per plot was 20m². Total number of plots: 15.
7. Planting date: 13 April 2001
8. Background of experimental plot: Moderate fertility, sandy loam. The previous crop was cotton.

Table 1 Treatment method for use of biocontrol agents

Treatment number	Treatment	Dosage used	Method	Source
1	X4	10x diluted	Seed dipped in suspension for 10 min.	
2	X4+BNR2	10x X4+5%BNR2	Seeds dipped in X4 solution for 10min, then coated with BNR2	Biological Control Laboratory, CAU
3	BNR2	5% (w/w)	Seed coating	
4	CK	water	Seeds dipped in water	
5	50% Carbendazol	1% (w/w)	seed coated with 1% carbendazol	XINXIN Pesticide Factory

Results

The germination rate of cotton seeds was recorded, and the fresh weight per hundred seedlings was measured at the time of disease rating. Disease ratings were made on May 17 and May 24. The results (Tables 2 and 3) showed that there was no significant difference between treatments at seedling stage. Table 3 presents the statistical analysis of the data reported in Table 2. We did not find BNR2 beneficial in reducing disease severity in the field, in contrast to the result that occurred in a previous greenhouse experiment.

We can see from Table 4 that there were clear yield responses to different treatments. This was similar to the result obtained in 2000. BNR2 treatment was beneficial in increasing yield.

Cotton yield averages (see also Table 4):

Treatment X4	3336.3	g/plot (i.e. 12 m ²)	
Treatment X4+BNR2	4240	g/12 m ²	(26.7% increase)
Treatment BNR2	4055	g/12 m ²	(21.2% increase)
Treatment Carbendazol	4342.7	g/12 m ²	(29.7% increase)
Treatment CK	3347	g/12 m ²	

Table 2 Raw data obtained from field experiment on biocontrol of cotton seedling disease

Treatment	Replicate	Seed germination rate (%)	Fresh weight		Fresh weight	
			(g / 100 plants) 17 May	DI, 17 May	(g / 100 plants) 24 May	DI, 24 May
X4	R1	34	56.7	41.7	105.1	36.5
X4	R2	29	56.7	30.0	103.4	39.8
X4	R3	32	56.7	44.2	83.3	28.3
X4+BNR2	R1	35	63.3	55.0	88.0	41.0
X4+BNR2	R2	38	73.3	30.8	105.1	42.4
X4+BNR2	R3	32	58.1	37.9	95.0	38.1
BNR2	R1	22	66.7	35.0	108.2	50.0
BNR2	R2	30	58.6	44.8	108.8	53.7
BNR2	R3	37	73.3	62.5	97.9	53.6
CK	R1	46	76.7	49.2	106.0	40.5
CK	R2	31	66.7	31.7	116.3	39.3
CK	R3	38	54.8	32.3	107.7	43.6
Carbendazol	R1	35	53.3	9.2	89.1	38.6
Carbendazol	R2	37	56.7	19.2	103.2	38.7
Carbendazol	R3	41	53.3	14.2	94.9	30.5

Table 3. Analysis of effectiveness of treatments on germination of seed and disease control

treatment	germination rate of seed	fresh weight (g) of 100 seedlings 17, May	DI May 17			Fresh weight(g) of 100 seedlings May 24	DI May 24		
			DI	0.05 0.01			DI	0.05 0.01	
X4+BNR2	35.0a	64.9a	41.2	a	BA	96.0b	40.5	b	B
X4	31.7a	56.7a	38.6	a	BA	97.2b	34.9	b	B
Carbendazol	37.7a	54.4a	14.2	b	B	95.7b	35.9	b	B
CK	38.3a	66.1a	37.7	a	BA	110.0a	41.1	b	B
BNR2	29.7a	66.2a	47.4	a	A	105.0ba	52.4	a	A

Table 4 Yield in response to treatment

TREATMENT	REPLICATE (g)			MEAN (g)	Significance	
	1	2	3		0.05	0.01
X4	4281	3634	2094	3336	a	A
X4+BNR2	2834	6252	3634	4240	a	A
BNR2	4281	4558	3326	4055	a	A
CK	3634	3604	2803	3347	a	A
50% Carbendazol	4928	4466	3634	4343	a	A

Note: Method of collecting yield data: There were 5 rows of cotton plants in each plot. Yield data were collected from the middle three rows. Total area for measuring yield was 12m² for each plot. Yield averages are reported in the text.

**Field experiments on biocontrol of seedling diseases
by binucleate Rhizoctonia in 2001**

Zhengzhou, Henan province

The Materials and Methods used in field testing at Zhengzhou were the same as at Baoding.

The results showed that all treatments reduced disease severity and increased fresh weight of seedlings, particularly after treatment with BNR2 and X4 plus BNR2 which gave the best effect.

The yield data showed that treatments of BNR2 and X4 plus BNR2 clearly increased yield, by 27% and 34 % respectively (both significant at P = 0.05), although other treatments were beneficial to yield as well.

Table 1 Effect of different treatments on control of seedling diseases and seedling growth of cotton

Treatment	Replicate	Number of plants	Disease incidence %	Disease index	Average DI	Control Effect (%)	Weight per seedling (g)	Average weight per seedling (g)	% change relative to CK
X4	1	24	100.0	58.3	54.5	12.2	0.83	0.92	+15.0
	2	31	100.0	50.5			0.96		
	3	59	94.9	54.8			0.96		
X4+BN R	1	56	80.4	43.4	45.8	26.2	1.16	1.19	+48.75
	2	56	89.3	46.4			1.15		
	3	40	85.0	47.5			1.25		
BNR	1	70	95.7	47.2	49.4	20.5	1.06	1.20	+50.0
	2	74	86.5	46.8			1.17		
	3	82	95.1	54.1			1.38		
Carben- dazol	1	5	100.0	53.3	55.3	11.0	0.72	0.85	+6.25
	2	53	98.1	57.2			0.87		
	3	38	97.4	55.3			0.97		
CK	1	29	96.6	62.1	62.1	--	0.69	0.80	--
	2	38	100.0	63.2			0.80		
	3	117	100.0	61.0			0.92		

Table 2 Effect of different treatments on yield of cotton

Treatment	Replicate	Yield per plot (kg)	Average yield per plot (kg)	% change relative to CK
X4	1	18.5	19.7	+17.3
	2	18.0		
	3	22.5		
X4+BNR	1	19.3	22.5*	+33.9
	2	25.3		
	3	23.0		
BNR	1	20.0	21.3*	+26.8
	2	22.5		
	3	21.5		
Carbendazol	1	16.1	18.4	+9.5
	2	20.2		
	3	19.0		
CK	1	14.0	16.8	--
	2	15.5		
	3	21.0		
lsd (0.05)			3.71	
			* significant increase	

Greenhouse experiments

A. Testing the effectiveness of binucleate *Rhizoctonia* on control of seedling diseases of cotton in a pot experiment.

In order to evaluate the effect of binucleate *Rhizoctonia* strains on the control of seedling diseases of cotton, a pot experiment was done in a greenhouse on the campus of CAU, Beijing.

Materials and methods

1. Soil: loam, pH 8.5, sterilized in autoclave.
2. Inoculation of soil by pathogen (*Rhizoctonia solani*): The pathogen was grown on bran medium for a week at 25C. Bran inoculant was incorporated into sterilized soil at 0.05% (w/w soil).
3. Application of biocontrol agents: Seeds of cotton were coated with biocontrol agents at 2% (w/w seed). Fungicide treatment was not included in this experiment. A healthy check was included. There were 5 replicate pots for each treatment, with 5 seeds planted in each pot. The whole experiment was repeated once.
4. Rating the disease severity was done 10 days after planting.

Results

The data in Table 4 show that there was no substantial difference in disease incidence between treated and untreated groups. The disease pressure was extremely high, probably because the experiment was done in autoclaved soil. Because of the severe disease it was not surprising that the biocontrol treatments could not reduce disease caused by *R. solani*.

Table 4 Testing effect of biocontrol agents on the control of seedling disease of cotton

	Treatment and disease incidence (%)							
	<i>BNR1</i> *	<i>BNR2</i> *	<i>BNR1</i> ⁺	<i>BNR2</i> ⁺	BM	CPf-10	CK (with pathogen)	CK (no pathogen)
Disease incidence (%)	88	92	92	80	100	88	96	20

Note: *BNR**, inoculant was made using agar; *BNR*⁺, inoculant was made as a powder.

B. Testing the effectiveness of different food bases and dosages of BNR strains on the control of seedling diseases of cotton

Materials and Methods

In order to find a suitable food base and dosage for the use of BNR to control disease, corn seed, wheat seed and millet seed were chosen as food substrates and used at different dosages. Cotton residue, wheat bran, residue of sugar beet, rice straw, wheat straw and sugar cane residue were chosen as candidates for an inexpensive food base.

In the pot experiment, the pathogen, *Rhizoctonia solani* was inoculated into soil as follows: *Rhizoctonia solani* was inoculated on wheat bran and incubated for more than one week. The culture was inoculated into autoclaved soil at a ratio of 1:2000 (w/w). Two cotton seeds were planted in each pot, and at the same time two cereal grains which had been colonized by BNR strains were buried beside the cotton seeds. (In this experiment, only cereal grains were used, no other substrate was used). Disease severity rating was measured when disease occurred. From the results of several preliminary experiments, we found that *BNR2* treatment could significantly reduce seedling death after cotton germination, but seed germination was relatively poor. On the other hand, *CPF-10* treatment improved the germination of cotton seed, but seedling death after germination was relatively high. We therefore concluded that it

might be better to inoculate cotton seeds with both of these biocontrol agents, to protect cotton seeds both during and after germination.

In order to find a cheaper food base for the production of BNR strains, the different types of plant residues were autoclaved, then inoculated by BNR. Growth rate and quantity of BNR2 produced on the various residues were investigated.

Results

Comparing the effect of food base as growth substrate for BNR

Cotton residue is the best food base in this experiment, and wheat bran was the second-best, followed by sugar beet residue. Rice straw, wheat straw and sugar cane residue were not suitable as a food base for the growth of BNR strains.

Control of disease as influenced by food base

In order to find a suitable food base and dosage for the use of BNR to control disease, corn seed, wheat seed and millet seed were chosen as food substrates and used at different dosages. Cotton residue, wheat bran, residue of sugar beet, rice straw, wheat straw and sugar cane residue were chosen as candidates for an inexpensive food base.

Food base: corn seed

Treatment	Number of seed	Number of seedlings (9 d after sowing)	% seedling survival (9 d after sowing)	% seedling survival (22 d after sowing)
BNR1+CPF-10	24	11	46	8
BNR2+CPF-10	24	8	33	25
CPF-10	24	7	29	0
CK	24	11	46	0

Food base: wheat seed

Treatment	Number of seed	Number of seedlings (9 d after sowing)	% seedling survival (9 d after sowing)	% seedling survival (22 d after sowing)
BNR1+CPF-10	24	11	46	4
BNR2+CPF-10	24	14	58	50
CPF-10	24	14	58	13
CK	24	12	50	0

Food base : millet seed

Treatment	Number of seed	Number of seedlings (9 d after sowing)	% seedling survival (9 d after sowing)	% seedling survival (22 d after sowing)
BNR1+CPF-10	24	6	25	17
BNR2+CPF-10	24	7	29	13
CPF-10	24	16	67	33
CK	24	13	54	0

CK without inoculation of pathogen

Treatment	Number of seed	Number of seedlings (9 d after sowing)	% seedling survival (9 d after sowing)	% seedling survival (22 d after sowing)
Wheat seed	24	8	33	33
Corn seed	24	6	25	38
Millet seed	24	5	21	21

Conclusion

Based on the result obtained from experiments in laboratory, greenhouse, field tests conducted at three provinces, it showed that BNR treatment always gave a significant increase in cotton yield where it was possible to make a measurement. However, there was no strong evidence for reduction in disease severity in the field with the formulation of BNR used.

Growth promotion of seedlings by binucleate *Rhizoctonia* has been reported several times in the literature, so it is possible that BNR could stimulate the growth of cotton in the absence of disease. It is also possible that the dosage of BNR formulation was not high enough, because the type of formulation can affect control of seedling diseases. The BNR2 strain is a very promising agent for increasing the yield of cotton. The production of BNR in a formulation is likely to be quite economical so that BNR could be produced in commercial quantities for the treatment of cotton seed prior to sowing.

Working group report to ACIAR

Since the extended project arising from “ACIAR P9680” was approved, a working group was established. At China Agricultural University, Tang Wenhua, Wang Ye, Zhang Liqun and Yang Hetong are involved in the group. The core research in the project is to evaluate the effectiveness of BNR strains in controlling seedling disease of cotton. The working group received the binucleate *Rhizoctonia* strains in April, 2000. Since then the two strains have been tested in laboratory, greenhouse and field plots for two years in three provinces in China. In order to strengthen this research, we invited Ms Yan Xioxue to coordinate the experiments conducted in Xinjiang. Dr. Li Hong-lian coordinated the experiments in Henan and Dr. Ma Ping was responsible for the experiments conducted in Hebei province. The purpose of the experiments in first year was to compare binucleate *Rhizoctonia* with biocontrol agents that were already available to assess the effectiveness of the fungal strains for disease control in the field. The purpose of the experiments conducted in second year (2001) was to reconfirm the effect of BNR strains on increasing yield. In the first year, we were not able to collect yield data at the Baoding experimental site. In the second year, we could not collect yield data at the Xinjiang experimental site due to damage to the plants caused by extremely cool conditions.

Based on the results obtained from experiments conducted over a two-year period, we recognized that isolate BNR2 is a promising biocontrol agent for increasing yield of cotton. We have contacted the LIENXIAN Company located in Qinghuang Dao city for a small amount of formulation of this fungus, so that we can conduct further experiments. The manager of this company expressed an interest in further developing this technique. Because this fungal strain was supplied by Dr. Maarten Ryder, CSIRO, we will discuss plans for further development with him.

Commercialization of *Trichoderma* for biological control of root diseases

Because our attempts to contact Dr. Niu Zhanguang, who was the manager of MINFUN Company, have failed, the commercialization of Tk7a has been stopped at present. Also, the tentative registration number of *Trichoderma* has lapsed. (The preliminary registration is valid for two years). If we take action to register the agent again, we have to re-apply for the preliminary registration.

We have contacted Dr. Ryder to ask for a new strain of *Trichoderma* which is a very good spore producer. If we have this strain that would be beneficial to industry production. Dr. Ryder agreed to supply this strain. Also, the Beijing-Goettingen Biotech Company may consider developing this agent for commercial use. In that case, a contract should be made between CAU and the new company.

APPENDIX 4

Publications (abstracts, short papers)

Please see original hard copy of report

APPENDIX 5

Report on visit to China

Dr Paul Harvey and Ms Rosemary Warren

Oct – Nov 2001

Increasing Crop Production through Biological Control of Soil-borne Root Diseases.

Report of visit to collaborating researchers in P. R. China, Oct. 29th - Nov. 20th 2001

Visiting Researchers:

Dr. Paul Harvey and Mrs. Rosemary Warren, CSIRO Land and Water (CLW), Adelaide, S.A.

Professor Tang Wenhua, Department of Plant Pathology, China Agricultural University (CAU), Beijing.

Oct. 30th – Nov. 2nd 2001

Major Activities:

1. Discussions pertaining to AUSAID project proposal:

International Workshop on Cereal Root Disease Diagnostics, Inner Mongolia, P. R. China Meeting (Tues. Oct. 30th) was held with Mr. Chris Brittenden (ACIAR country representative for P. R. China) to discuss the proposed research. Collaborating researchers at University of Inner Mongolia (Prof. Li Rong-Xi & Mr. Zhou Hong-Yu) have offered to help organise the workshop.

2. Discuss commercialisation of *Trichoderma koningii* (TK7a) for bio-control of root diseases.

The initial 2-year registration of TK7a for control of *Rhizoctonia cerealis* & take-all has passed. Since the dissolution of the original Minfeng Company the original commercialisation agreement been cancelled. TK7a needs to be re-registered for commercial use. The Beijing-Goettingen Biotech Company has been approached & has expressed interest in developing this agent for commercial use.

3. Prospects for using the *G. graminis*-specific probe for take-all detection in P. R. China

Meetings were held to discuss the potential incorporation of molecular diagnostic tools for root pathogenic fungi in the proposed International workshop on Cereal root Diseases. Discussions centred on testing CLW's *G. graminis* -specific DNA probe for detection and quantification of take-all disease in cereal cropping regions of China.

4. Presentations by researchers involved in ACIAR- and CAU- funded projects on bio-control of root diseases (cereals and cotton) and post-harvest diseases (horticulture).

Prof. Tang presented research on a recently patented (CAU) *Pseudomonas* strain used to control bacterial wilt diseases of Solanaceous horticultural crops and field results (Xinjiang, Hebei and Henan provinces) showing significant control of seedling diseases of cotton by binucleate *Rhizoctonia* spp. (ex. CLW).

Attended research seminars presented by Dr. Zhang Liqun and Dr. Wang Ye on molecular approaches to identifying mechanisms involved in the biological control of root diseases by rhizosphere bacteria.

Meetings were also held with post-graduate students (Ms. Zhang Yonghong, Ms. Niu Canfang and Ms. Wang Ying) to discuss their research on bio-control of root diseases and post-harvest diseases.

5. Visited Dr. Wang Xiaoming (Chinese Academy of Agricultural Science) and examined greenhouse trials for bio-control of *Pythium* and *Rhizoctonia* diseases of vegetables.

6. Dr. Harvey presented a public seminar in the Office of International Affairs (CAU) entitled:

Prospects for managing root disease complexes in cropping systems - The molecular ecology of plant-pathogen interactions.'

Dr. Yang Hetong, Biotechnology Research Centre, Shandong Academy of Science (SDAS), Jinan.

Nov. 3rd - Nov. 7th 2001

Major Activities:

1. Discussions pertaining to developing collaborative research proposals between SDAS, CAU and CLW, with particular emphasis on biotechnological approaches for identification and genetic improvement of beneficial bacteria and fungi for bio-control of root diseases. Issues associated with the commercialisation of bio-control strains developed in the ACIAR project were discussed and potential sources of funding for further product development were identified.
2. Presentations by researchers involved in ACIAR- and SDAS- funded projects on bio-control of root diseases (cereals and vegetables).

Dr. Hetong and Dr. Zhang Liqun (visiting from CAU) gave a presentation outlining their 'Disease Control Biotechnology' grant proposal to be submitted to the central government.

Discussions were held with Dr. Shi Jianguo and Dr. Li Mei regarding their research on biosensors and prospects for applying this technology for detection and tracking of soil-borne pathogens and their bio-control agents.

Meetings were held with post-graduate students to discuss their research on genetic engineering of *Bacillus* spp. to improve their bio-control efficacy (Ms. Huang Yujie) and molecular identification of *Trichoderma* species with bio-control (mycoparasitic) potential (Mr. Liao).

3. Toured laboratory, tissue culture and glasshouse facilities at the Biotechnology Research Centre of Shandong Academy of Science.
4. Visited Prof. Ding Ai Yun (Shandong Agricultural University, Taian), toured laboratory facilities and held discussions with post-graduate students on aspects of their research. Bio-control projects included mechanisms associated with the control of *Alternaria* spp. by the mycoparasite *Olpiarichum eenellum*, genetic and pathogenic diversity of *Rhizoctonia solani* and molecular taxonomy of *Bipolaris* spp.
5. Presented public seminars in Shandong Academy of Science.

Dr. Paul Harvey:

Prospects for managing root disease complexes in cropping systems - The molecular ecology of plant-pathogen interactions.

Mrs. Rosemary Warren:

Biological control of take-all and Rhizoctonia root rot of wheat in southern Australia.

Professor Zhang Bingxin, Department of Plant Protection, Zhejiang University (ZU), Hangzhou.

Nov. 8th - Nov. 18th 2001

Major Activities:

1. Discussions pertaining to developing collaborative research proposals between ZU, SDAS and CLW. Emphasis was placed on formulation technologies for bio-control agents and molecular approaches for studying the ecology of pathogens and bio-control agents in the rhizosphere. Methods and activities associated with the formulation, field testing and subsequent commercialisation of bio-control strains developed in the ACIAR project were discussed.

2. Sourced materials and taught techniques required for the formulation and field application of bacterial bio-control strains.

Bacillus strains that showed high disease control efficacy in pot trials were selected for formulation. Procedures were taught to post-graduate students and a suitable quantity of a test strain (PEP231) was prepared for a pot and glasshouse-based field trial against damping-off diseases (*Pythium* and *Rhizoctonia*). Design of the trials was discussed and finalised with the treatments including appropriate controls (water and fungicide checks) and two formulated strains (PEP231 and PEP66) being applied individually and as a mixture.

3. Presentations by researchers involved in ACIAR- and ZU- funded projects on bio-control of root diseases (cereals and vegetables).

Prof Zhang presented a comprehensive overview of ACIAR- and ZU- funded bio-control research projects. Current emphasis is on the formulation and commercialisation of strains that have showed significant disease control efficacy in glasshouse trials.

Meetings were held with post-graduate students to discuss their research. Projects included genetics and bio-control of *Pythium ultimum* (Lou Binggan), bio-control of damping-off diseases of vegetables (Zhang Zhen), formulation of bio-control agents against damping-off (Shen Ai Hua), mechanisms involved in plant growth promotion by rhizosphere bacteria (Shang Yu Lie) and bio-control of *Fusarium* head blight of barley (Shen Wei Feng).

Discussions were held with Dr. Gao and colleagues in the Institute of Biotechnology regarding their use of Microarray and genomic mapping technologies to study pathogenesis-related gene expression (plant-pathogen interactions).

4. Toured laboratory and tissue culture facilities and held discussions with researchers in the Departments of Plant Protection and Biochemistry, Zhejiang University.
5. Presented public seminars in Department of Plant Protection, Zhejiang University.

Dr. Paul Harvey:

Prospects for managing root disease complexes in cropping systems - The molecular ecology of plant-pathogen interactions.

Genetic and pathogenic diversity in a population of *Pythium ultimum* parasitising vegetables: Implications for disease control.

Molecular methods for studying the Rhizosphere ecology of biological control agents.

Mrs. Rosemary Warren:

Biological control of take-all and *Rhizoctonia* root rot of wheat in southern Australia.

Formulation methodology for bacterial biological control agents.