FINAL REPORT TO PROTEIN RESEARCH TRUST

EFFECT OF SOIL RHIZOBIA ON THE SUCCESS OF SOYBEAN INOCULANT STRAIN WB74

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Executive Summary

Agricultural legumes are commonly inoculated with selected strains of (brady)rhizobia in the expectation that nitrogen fixation and crop yields will be increased. An important factor that may limit this response is competition for nodulation by naturally-occurring populations in soils. The relative size and N2-fixing effectiveness of such populations is critical. If an inoculated strain is greatly outnumbered it usually has little impact even if the field population is less effective. Repeated inoculation may not overcome the problem.

For example, negligible proportions of strain WB 1 were recently found in several South African populations although this strain had been applied in commercial soybean inoculants over a period of 20 years. A more effective inoculant strain WB74 was introduced in 1997 but its usefulness in established soybean production regions will depend on its ability to compete against and displace native bradyrhizobia.

The present study was undertaken three years after WB74 had replaced WB 1 to (a) determine the extent to which the new strain had established itself in soybean production regions, (b) characterise symbiotic, serological and genotypic properties of naturally-occurring populations of bradyrhizobia at selected sites and (c) evaluate response to inoculation with WB74 at these sites using different application methods (seed or liquid-in-furrow).

Most soils from ten localities sampled at the end of the 2000/2001 soybean season contained large rhizobial populations (>10^5cells g^{-1}). Isolates from each soil were identified serologically using antisera specific for WB74 as well as strains WB1, WB61, WB66 used in previous commercial inoculants. Soybean was planted for the first time at Amersfort and only strain WB74 was detected at this as well as two other localities (Bethal, Naboomspruit),. The remaining seven soils predominantly contained bradyrhizobia that reacted weakly with antiserum to strain WB66. In three of these (Lothair,Vryheid, Bergville A), WB74 comprised 5-35% of the bradyrhizobial isolates. However, this strain was not detected amongst isolates from the remaining four soils (Newcastle, Bergville B, Morgenzon, Koedoeskop). Observed variability in establishment of WB74 at the individual sites could have been due to different times of introduction of the new inoculant as well as differences in size and composition of natural

populations. Clearly, future monitoring will be essential to determine the degree to which WB74 can displace native populations of soybean bradyrhizobia.

Field trials were subsequently carried out during the 2001/2002 season at three sites Bergville (pH 5.16), Koedoeskop (pH 7.76) and Morgenzon (pH 6.33). These soils each contained populations of bradyrhizobia in which relatively small proportions of WB74 were detected (Bergville, 14%; Koedoeskop, 6.4%; Morgenzon, 15.6%). At each site, thirteen to fifteen isolates were obtained from nodules of uninoculated plants.

Inoculant strains and isolates were subjected to serological and genomic fingerprinting. Several were closely related to *Bradyrhizobium elkanii* serotypes 31 and 76. They included nine isolates from Bergville and three from Morgenzon as well as the discontinued inoculant strains WB1 and WB61, which were closely related to one another. DNA profiles of the *B. elkanii* isolate group were heterogeneous and it was difficult to ascribe origins to either inoculant strain. For example, four isolates were serologically indistinguishable from WB 1 but only two had genetic profiles closely related to this strain.

Several serotypes of *Bradyrhizobium japonicum* were identified amongst the isolates but none were related to WB66, a strain of *B. japonicum* used previously in inoculants. Whereas WB66 was taxonomically related to serotype strain USDA38, four isolates from Bergville were related to USDA6. In contrast, twelve isolates from Morgenzon were related to USDA123, a strain dominant in soils of the mid-west USA. Fifteen isolates from Koedoeskop were all related to USDA135, a serotype found in alkaline soils of the USA. The origin of the *B. japonicum* serotypes is not known although they may have been introduced in contaminated uninoculated seed.

Greenhouse experiments showed that 73% of the isolates from Koedoeskop were in the same N2-fixing effectiveness category as WB74, whereas only 40% of the isolates from Bergville and Morgenzon were in this group.

Populations of bradyrhizobia in soils used for inoculation trials at Bergville, Koedoeskop and Morgenzon were large (>10^4 cells g^{-1}). Nevertheless, inoculation significantly increased nodule occupancy by WB74 at Bergville and Morgenzon, three-fold higher occupancy levels being achieved using seed and normal (1x) liquid application methods. Use of a high (10x) liquid inoculation level at Bergville increased WB74 nodule occupancy five-fold to 69.6%, resulting in an appreciable (19.8%) yield increase compared to the non-inoculated control. Inoculation had no effect on nodule occupancy at Koedoeskop, however, irrespective of the method used. Soil pH may have been a factor as the alkaline Koedoeskop soil contained bradyrhizobia adapted to high pH.

The finding that all three soils contained bradyrhizobia unrelated to commercial inoculant strains was unexpected considering the 40 year history of inoculation and the relatively short period (about 50 years) during which soybean has been cultivated in South Africa. The soils differed in pH and other properties and probably contained bradyrhizobia adapted to each, as shown by the alkaline-tolerant serotype found at Koedoeskop. The effect of soil type and other environmental factors on WB74 inoculation success merits further investigation.