# Characterization of *Rhizoctonia solani* AG-8 from Bare Patches by Pectic Isozyme (Zymogram) and Anastomosis Techniques

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## ABSTRACT

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Isolates of *Rhizoctonia solani* AG-8 collected from Rhizoctonia bare patches at two locations in Western Australia were characterized by pectic isozyme (zymogram) and anastomosis techniques. All isolates collected from the same patch at the same time and at different times up to 10 mo apart were members of the same zymogram group. Anastomosis techniques demonstrated that these multiple isolates from the same patch were members of the same clone, as indicated by the clonal anastomosis reaction. Anastomosis reactions also indicated that isolates from the same zymogram group but from different patches in the same field or farm may or may not be members of the same clone. In general, patches with the same zymogram group and in close proximity to one another are more likely to be of the same clone than are patches separated by greater distances. Thus, the zymogram technique demonstrated the existence of distinct groups (currently five) within *R. solani* AG-8, and the anastomosis technique demonstrated there may be many different clones within each zymogram group. In general, the chance of isolates collected from patches caused by the same zymogram group being members of the same clone decreases as distance between patches increases. However, clonal relationships have been detected between isolates of the same zymogram group collected from patches separated by hundreds of kilometers in Australia and in one case in Oregon. Some possible explanations for the spread of clones are presented.

*Additional keywords*: anastomosis group.

Rhizoctonia bare patch disease of cereals, legumes, and mixed legume-grass pasture was first recorded in South Australia more than 60 yr ago (27,28). Since then, it has been recorded in most cereal-growing regions of Australia (7,8,12) and in association with cereals in other parts of the world (19,31). The "root attacking" (5,9) or patch-forming (30) strains of *Rhizoctonia solani* Kühn (teleomorph *Thanatephorus cucumeris* (A.B. Frank) Donk) are now known to comprise a unique anastomosis group (AG) of *R. solani*, designated AG-8 (1,21,22,26).



At least five distinct zymogram-pattern groups (ZG) exist within *R. solani* AG-8: ZG1-1, -2, -3, -4, and -5 (16,20,29,30). ZG1-5 (16) initially was designated as ZG2 (30). Additionally, MacNish and Sweetingham (17) established that from each distinct bare patch only a single ZG of *R. solani* AG-8 can be isolated, and they proposed that each patch emanates from a single infection focus. They also established that only *R. solani* AG-8 is responsible for Rhizoctonia bare patch disease, whereas isolates of other AGs of *R. solani*, as well as other *Rhizoctonia* species, may cause varying levels of root rot of cereals without producing the characteristic bare patches.

TABLE 1. A summary of anastomosis reactions between isolates of *Rhizoctonia solani* AG-8 from individual bare patches collected on four occasions from five patches on Esperance Downs Research Station, WA, Australia

| Patch number[a] | Zymogram group | Isolate numbers and anastomosis reaction | | | |
|---|---|---|---|---|---|
| | | Jan. 18[b] | Feb. 15 | Mar. 1 | Oct. 13 |
| 42 | ZG1-1 | 91019 | 91053, C3 | 91097, C3 | 91544, C3 |
| 85 | ZG1-5 | 91028 | 91063[c], – | 91108, C3 | 91553, C3 |
| 87 | ZG1-5 | 91030 | 91069, C3 | 91119, C3 | –[d], – |
| 91 | ZG1-5 | 91002 | 91042, C3 | 91090, C3 | 91519, C3 |
| 92 | ZG1-4 | 91006 | 91049, C3 | 91094, C3 | 91529, C3 |

[a]Patch 42 in lupins, 85 and 87 in triticale, and 91 and 92 in barley.
[b]Date isolates collected.
[c]Not available—isolate dead.
[d]Not available—no isolate obtained because patch disappeared.

TABLE 2. A summary of anastomosis reactions among isolates of *Rhizoctonia solani* AG-8 collected on 18 January 1991 from five bare patches on Esperance Downs Research Station, WA, Australia

| Patch number[a] | Zymogram group | Isolate number | Isolate numbers | | | | |
|---|---|---|---|---|---|---|---|
| | | | 91019 | 91028 | 91030 | 91002 | 91006 |
| 42 | ZG1-1 | 91019 | C3 | C2 | C2 | C2 | C2 |
| 85 | ZG1-5 | 91028 | – | C3 | C3 | C3 | C2 |
| 87 | ZG1-5 | 91030 | – | – | C3 | C3 | C2 |
| 91 | ZG1-5 | 91002 | – | – | – | C3 | C2 |
| 92 | ZG1-4 | 91006 | – | – | – | – | C3 |

[a]Patch 42 in lupins, 85 and 87 in triticale, and 91 and 92 in barley.

There have been several reports that define and categorize the anastomosis reactions occurring between opposing hyphae of isolates of *R. solani* (6,18,24). Carling et al (2) developed and refined the interpretation of the anastomosis reaction, giving a detailed description of the interaction observed in a series of four categories. These categories range from C0, in which no reaction occurs, to C3, in which walls and membranes of anastomosing hyphae fuse and the cytoplasm of anastomosing hyphae intermingles. The C3 reaction is comparable to the perfect reaction described by Matsumoto et al (18) and to the S reaction described by Flentje and Stretton (6). The C3 reaction would be expected to occur in self-anastomosis as well as between hyphae of any two isolates of the same clone.

Matsumoto et al (18), Flentje and Stretton (6), and Parmeter et al (24) all refer to the type of anastomosis reaction called C3 by Carling et al (2) as the characteristic reaction of self-anastomosis. Thus, the C3 reaction may be considered evidence of a clonal relationship between isolates. Ogoshi and Ui (23) working with isolates of *R. solani* AG-1, -2-2, and -3 collected from different plants and fields of rice, potatoes, and sugar beets, respectively, demonstrated that the incidence of clonal relationships between isolates varied as the distance between collection sites varied. We believe that anastomosis reactions can be used to determine clonal relationships in all AGs of *R. solani*, including AG-8.

In this paper we use the anastomosis technique to test the hypothesis that multiple isolates representing a single ZG of *R. solani* AG-8, made from a bare patch on one or more occasions during periods of up to 10 mo, are members of the same clone. We also test the hypotheses that isolates of the same ZG collected from different patches may or may not be clones and that isolates from different ZGs of AG-8 will not be clones.

## MATERIALS AND METHODS

**Isolate descriptions and sources.** Descriptions of the isolates used in this study are summarized in Tables 1–6. All isolates from Western Australia (WA) were obtained using undisturbed soil cores baited with wheat seedlings (13). A variety of other collection methods were used to obtain isolates from South Australia (SA), New South Wales (NSW), Australia, and Oregon and Washington, United States. Descriptions of all bare patches used as sources of isolates of *R. solani* AG-8 for this study are summarized in MacNish and Sweetingham (17). Brief descriptions of the patches will be given with each experiment.

TABLE 3. Summary of anastomosis reactions among isolates of ZG1-2 *Rhizoctonia solani* AG-8 collected from 14 discrete bare patches in a lupine field near Kojaneerup, WA, Australia[a]

| Base patch no. | Base isolate no. | Nearby patches | | Distant patches | |
|---|---|---|---|---|---|
| | | Patch no. | Isolate no. | Patch no. | Isolate no. |
| 115 | 90798 | 111 | 90771 | 103 | 90742 |
| | | 112 | 90765 | 107 | 90745 |
| | | 113 | 90781 | 108 | 90755 |
| | | 114 | 90790 | 123 | 90835 |
| | | 116 | 90803 | 127 | 90838 |
| | | 117 | 90812 | | |
| | | 118 | 90817 | | |
| | | 119 | 90825 | | |

[a]The isolate from each patch yielded a C3 anastomosis reaction with the isolate from patch 115.

**Zymogram-group typing.** The zymogram identifications were made electrophoretically, using culture fluids run on pectin-acrylamide gels (30).

**Anastomosis techniques.** All isolates were grown on rehydrated potato-dextrose agar (PDA) prior to use in anastomosis tests. The method was based on that of Parmeter et al (24) as used by Carling et al (4). Autoclaved rectangles of cellophane (P. D. Cellophane, Du Pont Plastics, E. I. Du Pont De Nemours & Co., Inc., Wilmington, DE) were dipped in PDA (13 g/L) and placed on water-agar plates, and a rectangle (0.5 × 1 cm) of freshly grown test culture was placed at each end of the cellophane rectangle. Cultures were grown at room temperature until the cultures overlapped. The zone of confrontation was stained with 0.05% trypan blue in lactophenol and examined at 100× magnification. Confirmation of category of anastomosis reaction was established with 400× magnification. The presence of five or more anastomosis points in the highest category observed placed the isolate pair in that category.

Detailed descriptions of the categories of anastomosis reactions in *R. solani* (3) are given in Table 7. "Fuse" or "fusion" is used to describe only C3 anastomosis. Also, for an anastomosis point to qualify as indicating a clonal relationship between isolates, self-anastomosis is set as the standard. Thus, in tip-to-tip anastomoses, there should be little or no evidence of the specific location of the anastomosis point. In tip-to-side of hyphae anastomoses, there should be no evidence of a wall and little or no constriction at the anastomosis point. In side-to-side anasto-

moses, there should be no evidence of a wall, and the connecting channel should be approximately the same diameter as the anastomosing hyphae. No doubtful C3 anastomosis points (including those with cell death) were counted, and the search continued until five C3 anastomoses fitting the above criteria were obtained. Whenever unexpected reactions occurred, retesting was done to confirm the original result.

**Multiple collection times from five patches.** Multiple isolates were collected on four occasions (18 January, 15 February, 1 March, and 13 October 1991) from five patches on the Esperance Downs Research Station (EDRS) 35 km north of Esperance, WA, Australia. The patches sampled were in lupines (*Lupinus angustifolius* L.; patch 42), triticale (× *Triticosecale* Wittm.; patches 85 and 87), and barley (*Hordeum vulgare* L.; patches 91 and 92). Patch 42 was 75 m from patch 85, which in turn was 25 m from patch 87. Patches 91 and 92 were 10 m apart in a field 1.7 km northeast of patch 42.

The ZG affiliation was determined for all isolates, and a single isolate from each patch for each sampling time was selected for AG determination. One isolate from the first sampling time was tested against one isolate from the three remaining sampling times (Table 1). These tests were done to determine the clonal relationship among isolates removed from the same patch over time.

Five isolates, one isolate from the earliest collection date

TABLE 4. A summary of anastomosis reactions for the comparison of isolates of ZG1-1 *Rhizoctonia solani* AG-8 collected from patches 135, 136, or 137 on Esperance Downs Research Station (EDRS), WA, Australia, with other isolates of ZG1-1 from the same or a close by patch and isolates from other locations

| | | Patch 135 | | | Patch 136 | | | Patch 137 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Comparison | Collection date | Patch no. | Isolate no. | Category | Patch no. | Isolate no. | Category | Patch no. | Isolate no. | Category |
| Self-anastomosis | 30 Oct. 91 | 135 | 91503 | C3 | 136 | 91508 | C3 | 137 | 91512 | C3 |
| Other isolates from same patch | 30 Oct. 91 | 135 | 91504 | C3 | 136 | 91509 | C3 | 137 | 91514 | C3 |
| | 30 Oct. 91 | 135 | 91505 | C3 | 136 | 91510 | C3 | 137 | 91515 | C3 |
| | 30 Oct. 91 | 135 | 91506 | C3 | 136 | 91511 | C3 | 137 | 91516 | C3 |
| Other isolates from same patch, later dates | 21 Nov. 91 | 135 | 91638 | C3 | 136 | 91655 | C3 | 137 | 91650 | C3 |
| | 16 Dec. 91 | 135 | 91838 | C3 | 136 | 91844 | C3 | 137 | 91860 | C3 |
| | 14 Jan. 92 | 135 | 92001 | C3 | 136 | 92006 | C3 | 137 | 92008 | C3 |
| | 16 Jan. 92 | 135 | 92011 | C3 | 136 | 92014 | C3 | 137 | 92015 | C3 |
| | 21 Jan. 92 | 135 | 92024 | C3 | 136 | 92029 | C3 | 137 | 92033 | C3 |
| | 11 Feb. 92 | 135 | 92278 | C3 | 136 | 92279 | C3 | 137 | 92280 | C3 |
| | 18 Feb. 92 | 135 | 92281 | C3 | 136 | 92282 | C3 | 137 | 92283 | C3 |
| Isolates adjacent patches | 30 Oct. 91 | 136 | 91508 | C3 | 135 | 91503 | C3 | 135 | 91503 | C3 |
| | 30 Oct. 91 | 137 | 91512 | C3 | 137 | 91512 | C3 | 136 | 91508 | C3 |
| Isolates from other patches on EDRS | 29 Aug. 90 | 22 | 90291 | C2 | 22 | 90291 | C2 | 22 | 90291 | C2 |
| | 18 Jan. 91 | 42 | 91019 | C2 | 42 | 91019 | C2 | 42 | 91019 | C2 |
| | 15 Feb. 91 | 42 | 91053 | C2 | 42 | 91053 | C2 | 42 | 91053 | C2 |
| | 1 Mar. 91 | 42 | 91097 | C2 | 42 | 91097 | C2 | 42 | 91097 | C2 |
| | 13 Oct. 91 | 42 | 91544 | C2 | 42 | 91544 | C2 | 42 | 91544 | C2 |
| | 5 April 91 | S7 | 91198 | C2 | S7 | 91198 | C2 | S7 | 91198 | C2 |
| Isolates from other patches WA[a] | 3 Aug. 89 | 4 | 89570 | C3 | 4 | 89570 | C3 | 4 | 89570 | C3 |
| | 30 Aug. 90 | 61 | 90410 | C2 | 61 | 90410 | C2 | 61 | 90410 | C2 |
| Isolates from patch in SA or NSW | 1990 | – | SA 32 | C2 | – | SA 32 | C2 | – | SA 32 | C2 |
| | 1990 | – | SA 49 | C2 | – | SA 49 | C2 | – | SA 49 | C2 |
| | 1991 | – | SA 67 | C2 | – | SA 67 | C2 | – | SA 67 | C2 |
| | 1991 | – | SA 81 | C2 | – | SA 81 | C2 | – | SA 81 | C2 |
| | 1991 | – | SA 103 | C2 | – | SA 103 | C2 | – | SA 103 | C2 |
| | 1991 | – | SA 108 | C2 | – | SA 108 | C2 | – | SA 108 | C2 |
| | 1991 | – | SA 124 | C2 | – | SA 124 | C2 | – | SA 124 | C2 |
| | – | – | NSW R11 | C2 | – | NSW R11 | C2 | – | NSW R11 | C2 |
| | – | – | NSW R16 | C2 | – | NSW R16 | C2 | – | NSW R11 | C2 |
| | – | – | NSW R18 | C2 | – | NSW R18 | C2 | – | NSW R18 | C2 |
| Isolate from Oregon | – | – | CB-01 | C2 | – | CB-01 | C2 | – | CB-01 | C2 |

[a] WA = Western Australia; SA = South Australia; and NSW = New South Wales.

TABLE 5. A summary of anastomosis reactions among isolates of ZG1-2 *Rhizoctonia solani* AG-8 collected from Australia and the United States

| | | Western Australia | | | South Australia | | | Washington | |
|---|---|---|---|---|---|---|---|---|---|
| Origin | Isolate no. | 91439 | 89662 | 90798 | SA 45 | SA 50 | SA 88 | C1 | H1 |
| Esperance, Aust. | 91439 | C3 | C2 | C2 | C2 | C2 | C2 | C2 | C2 |
| Circle Valley, Aust. | 89662 | – | C3 | C2 | C2 | C2 | C3 | C2 | C2 |
| Kojaneerup, Aust. | 90798 | – | – | C3 | C2 | C2 | C2 | C2 | C2 |
| Lowbank, Aust. | SA 45 | – | – | – | C3 | C2 | C2 | C2 | C2 |
| Pinnaroo, Aust. | SA 50 | – | – | – | – | C3 | C2 | C2 | C2 |
| Cooke Plain, Aust. | SA 88 | – | – | – | – | – | C3 | C2 | C2 |
| Washington, U.S. | C1 | – | – | – | – | – | – | C3 | C2 |
| Washington, U.S. | H1 | – | – | – | – | – | – | – | C3 |

TABLE 6. A summary of anastomosis reactions between isolates of ZG1-1 *Rhizoctonia solani* AG-8 collected from Australia and the United States

| Origin | Isolate number | South Australia | | | | | New South Wales | | | Oregon |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 32 | 49 | 67 | 103 | 108 | R11 | R16 | R18 | CB-01 |
| Elliston, Aust. | SA 32 | C3 | C2 | C2 | C2 | C2 | C2 | C2 | C2 | C2 |
| Pinnaroo, Aust. | SA 49 | – | C3 | C2 | C2 | C2 | C2 | C2 | C2 | C2 |
| Lowbank, Aust. | SA 67 | – | – | C3 | C2 | C2 | C3 | C2 | C3 | C3 |
| Pinnaroo, Aust. | SA 103 | – | – | – | C3 | C2 | C2 | C2 | C2 | C2 |
| Workurna, Aust. | SA 108 | – | – | – | – | C3 | C2 | C2 | C2 | C2 |
| Parkes, Aust. | NSW R11 | – | – | – | – | – | C3 | C2 | C3 | C3 |
| Unknown, Aust. | NSW R16 | – | – | – | – | – | – | C3 | C2 | C2 |
| Manildra, Aust. | NSW R18 | – | – | – | – | – | – | – | C3 | C3 |
| Oregon, U.S. | CB-01 | – | – | – | – | – | – | – | – | C3 |

TABLE 7. Categorization of anastomoses in *Rhizoctonia solani* that occur between hyphae[a]

| Category | Relatedness | Description of interaction |
|---|---|---|
| C0 | Not related (different anastomosis groups) | No interaction |
| C1 | Distantly related (different anastomosis groups or same anastomosis groups) | Contact between hyphae; apparent connection of walls but no evidence of wall penetration or membrane-membrane contact; occasionally one or both anastomosing cells and adjacent cells die |
| C2 | Related (same anastomosis groups, different clones) | Wall connection obvious; membrane contact uncertain; location of reaction site obvious; diameter of anastomosis point less than hyphal diameter; anastomosing and adjacent cells always die |
| C3 | Closely related (same anastomosis group), (same clone), (same isolate) | Walls fuse; membranes fuse; anastomosis point frequently not obvious; diameter of anastomosis point equal or nearly equal to hyphal diameter; anastomosing cells and adjacent cells may die but generally do not |

[a]Categorization based on reactions and cytological occurrences at the point of anastomosis between two hyphae (HYPHA-HYPHA reaction). These occurrences are observable at the light microscopic level. Text was created by Carling and Leiner, 1992.

(January 18) from each of the five patches described above, were anastomosed in all combinations (Table 2). This was done to determine whether there were any clonal relationships among isolates from the five patches described above.

**Interaction of isolates from many patches in one field at Kojaneerup.** A field at Kojaneerup (50 km northeast of Albany, WA, Australia) with no known history of Rhizoctonia bare patch was sown to barley in 1988 and 1989 and to lupines in 1990, using minimum tillage. In this context, minimum tillage means weed growth is killed with a desiccant herbicide and the crop is sown a day or two later with a single pass of a seeding drill equipped with tine-cultivators set at a depth of about 3 cm. Similar practices are known to exasperate Rhizoctonia bare patch disease (15,25,31).

In 1990, 45 instead of 41 patches were observed in the upper and northern third of the field (Fig. 1). Patches were not detected in the lower southern part of the field where the soil type was different. Isolates were collected from 20 of these patches (Fig. 1), and their ZGs were determined. Isolates from 13 patches were used in anastomosis tests. An isolate from patch 115 was paired with an isolate collected from each of eight surrounding patches (Table 3). The isolate from patch 115 also was paired with an isolate from each of five more distant patches (Table 3). All the tests were done to determine whether there were clonal relationships between isolates from the various patches in the field.

**Interactions of isolates from three adjacent patches.** For this study, three patches (135, 136, and 137) in a pasture of subterranean clover (*Trifolium subterraneum* L.) on EDRS were chosen. The patches were in a roughly triangular formation with centers about 10 m apart and were first observed at the end of the growing season (October). Isolates were collected on eight occasions over a 4-mo period during the summer, and their ZGs were determined. For each patch, the isolate collected first was anastomosed with other isolates collected from the same patch. The first isolate collected from each patch also was anastomosed with isolates collected from the two adjacent patches. This was

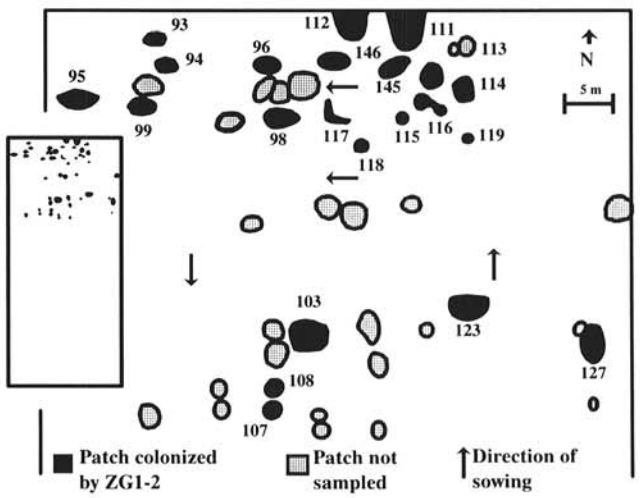


**Fig. 1.** Distribution of patches in a lupine crop at Kojaneerup, WA, Australia, in 1990 and 1991. Insert shows entire field.

done to determine whether the isolates removed over time from each patch were clones and to determine whether the three patches were colonized by the same or different clones.

The isolates collected during the first sampling time from patches 135, 136, and 137 also were tested against isolates from three other patches on EDRS, from patch 4 at Circle Valley (90 km north of Esperance), and from patch 61 at Scaddan (50 km north of Esperance). Seven isolates from South Australia (provided by P. J. L. Whittle), three isolates from New South Wales (provided by P. T. W. Wong), and one isolate from Oregon (provided by R. W. Smiley) were included in this test. Descriptions of the isolates used are summarized in Table 4. These tests were done to determine whether the isolates from the three patches

were clonally related to each other and to other ZG1-1 isolates from EDRS, other locations in Western Australia, more distant Australian locations, or Oregon.

**Interaction of ZG1-2 isolates from Western Australia, South Australia, and Washington.** Isolates from three locations in Western Australia, three in South Australia, and two in Washington (provided by D. M. Weller) were tested for anastomosis in all combinations. Details of the isolates are given in Table 5. These tests were performed to determine if clonal relationships existed between isolates of ZG1-2 from widely distributed locations.

**Interaction of ZG1-1 isolates from South Australia, New South Wales, and Oregon.** A random selection of five isolates from South Australia, three from New South Wales, and one from Oregon was tested for anastomosis in all combinations. Isolate details are summarized in Table 6. The reason for doing these tests was the same as that for ZG1-2.

## RESULTS

**Isolates from five patches.** All isolates from patch 42 were ZG1-1, all from patches 85, 87, and 91 were ZG1-5, and all from patch 92 were ZG1-4. The anastomosis tests with isolates from individual patches, for all five patches at all collection times, gave the C3 reaction (Table 1). When isolates were subjected to interpatch comparison, all isolates from patches 85, 87, and 91 gave C3 reactions (Table 2). The ZG1-1 and -4 isolates from patches 42 and 92, respectively, gave C2 reactions with each other and with all the ZG1-5 isolates from patches 85, 87, and 91 (Table 2).

**Kojaneerup.** All isolates collected from patches at Kojaneerup were ZG1-2, and all the combinations of isolates tested for anastomosis gave C3, the clonal reaction (Table 3).

**Isolates from three patches and other locations.** All the isolates in these tests (Table 4) were ZG1-1. The anastomosis tests on isolates from patches 135, 136, and 137 demonstrated that all tested combinations both within and between patches gave C3 reactions and, thus, were clones (Table 4). The reaction of isolates from patches 135, 136, and 137 with isolates from other ZG1-1 patches collected from EDRS, from other locations in Western Australia, or from other locations in South Australia, New South Wales, or Oregon, with three exceptions, gave C2 reactions. The three exceptions were C3 reactions between isolate 89570 × 91503, 91508, and 91512 (Table 4).

**Isolates from Western Australia, South Australia, and Washington.** All isolates used in this test (Table 5) were ZG1-2. Each isolate produced a C3 reaction when anastomosed with itself. As expected, most isolates produced a C2 reaction when anastomosed with isolates from the same or different regions. The one exception was isolate SA88 × 89662, a pairing that unexpectedly produced a C3 reaction.

**Isolates from South Australia, New South Wales, and Oregon.** All isolates in this test (Table 6) were ZG1-1. Once again, each isolate produced a C3 reaction when anastomosed with itself. Also, as expected, most isolates produced a C2 reaction when anastomosed with isolates from the same or other regions. However, several pairings involving isolates from New South Wales, South Australia, and Oregon produced C3 reactions although C2 was the expected reaction.

## DISCUSSION

Pectic isozyme (zymogram) technology (30) has permitted the subdivision of *R. solani* AG-8 into five (ZG1-1, -2, -3, -4, and -5) smaller, more homogeneous groups. Further, specific reading of anastomosis reactions permits identification of clonal relationships between individual isolates of *R. solani*. Ogoshi and Ui (23) were the first to report the anastomosis technique as a method for identifying clonal relationships between isolates of *R. solani*. Studying collections of isolates of *R. solani* AG-1, -2, and -3, they found isolates of the same AG from the same plant were more likely to be clonally related than were isolates from different plants. They also found that isolates from different fields were less likely to be clonally related than were isolates from the same field. Ogoshi and Ui (23) concluded that the probability of clonal relationships between isolates decreases as the distance between collection points increases.

MacNish and Sweetingham (17) found that isolates of *R. solani* AG-8 collected from discrete bare patches all are members of the same ZG. Data contained in this report indicate that multiple isolates collected from a patch over a period of time are members of a single clone of *R. solani* AG-8. This supports the hypothesis that discrete Rhizoctonia bare patches arise from a single infection focus. The apparent radial pattern of expansion of patches (G. C. MacNish, *unpublished data*), the isolation of only one ZG of *R. solani* AG-8 from each patch (17), and the clonal anastomosis reaction (C3) shown here all support the proposition that a single isolate spreading from a single infection focus is responsible for the patch.

In the interpatch comparison among the five patches (Table 2), the C2 anastomosis reaction between isolates from different ZGs was expected based on differences in zymogram reaction. The clonal relationship (indicated by the C3 anastomosis reaction) between the isolates of ZG1-5 from the three different patches was probable, especially for patches 85 and 87, which are located within meters of each other and easily could have resulted from the same introductory source. Patch 92 is more than 1.7 km from patches 85 and 87, and although possible, it is far less likely that it would come from the same source. However, because EDRS is a research station where there is considerable movement of personnel and equipment around the farm, it is probable that isolates could have been spread to many parts of the farm.

The demonstration that all isolates collected from bare patches at Kojaneerup were of a single zymogram group (ZG1-2) and a single clone suggests that there has been a spread of the original isolate by tillage. Elongation of patches in the direction of tillage has been reported (10,14), and the shape and pattern of the distribution of patches suggests that in this field patches are elongated in the direction of sowing (Fig. 1). At Kojaneerup, it appears the pathogen was introduced prior to 1990 without causing obvious patches. By 1990, expansion from original infection foci was sufficient for the patches to be obvious. The origin of the source of inoculum is not known.

The results from the comparison of three patches confirm the results from the comparison of five patches with multiple isolates from each patch being clonally related (Table 4). The demonstration that all three patches were caused by isolates of the same clone, though not expected in this case, is highly probable. All three patches are close together, approximately the same size, and essentially circular (there has been no recent cultivation to change the shape of the patches) in shape. These observations suggest all three were established at the same time.

The discovery of clonal relationships between isolates of the same ZG of AG-8 from widely separated parts of Australia, and in one incidence Oregon, was unexpected but not improbable. A clone of ZG1-1 was detected in three patches on EDRS and in a patch on a farm at Circle Valley, 90 km away (Table 4). On the same farm, an isolate of ZG1-2 from another patch is a clone of an isolate from Cooke Plain (100 km southeast of Adelaide, SA) (Table 5). Another isolate of ZG1-1 obtained from Lowbank, SA (140 km northeast of Adelaide), is a clone of two isolates from the region near Parkes, 280 km west of Sydney, NSW, and one isolate from Oregon (Table 6). Clones from within some ZGs of AG-8 clearly have been spread over long distances within Australia. Although this is somewhat unexpected, it is similar to the distribution of clones of *Sclerotinia sclerotiorum* over similar distances recently reported in Canada (11).

Presumably, the spread of these clones of *R. solani* was by physical means, such as the transport of soil or infected organic matter on machinery or other equipment. The cereal belt in New South Wales, Victoria, and South Australia is continuous, and thus, there would have been many opportunities for the inadvertent spread of the pathogen since it was discovered in Australia in the 1920s (7,27). The cereal belt in Western Australia is separated from the cereal-growing regions of the rest of Australia

by 1,000 km of the agriculturally barren Nullarbor Plain. However, when the Esperance region was first farmed in the 1950s and 1960s, there was a migration of many farmers with their equipment from South Australia to the region. There was also a major importation of medic (*Medicago* spp.) seed from South Australia to the Esperance region in the 1960s. Both activities would have provided opportunities for the accidental spread of the pathogen from South Australia to Western Australia, where it was first identified in the Esperance region in 1971 (12). We cannot explain the clone in Oregon, but the evidence indicates there has been an inadvertent interchange of *R. solani* AG-8 between Australia and Oregon on some occasion in the past.

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