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Anastomosis groups, pathogenicity and sensitivity to fungicides of *Rhizoctonia solani* isolates collected on potato crops in France

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Abstract

A collection of 241 isolates of *Rhizoctonia solani* obtained from potato plants grown in different areas in France was characterized for anastomosis grouping, symptomatology on tubers of different cultivars and sensitivity to three fungicides. Most isolates collected belonged to (anastomosis groups (AGs)) AG 3, but 2% and 4% of the isolates were AG 5 and AG 2-1. AG 3 and AG 2-1 isolates were mostly obtained from sclerotia on tubers, but all AG 5, some AG 3 and some AG 2-1 isolates were recovered from superficial tuber alterations, like deformations, corky or scabby lesions. Sclerotia were formed on tubers produced by healthy stem cuttings grown in soil artificially infested with AG 3, but not on tubers grown in soil infested with either AG 5 or AG 2-1. No variation in susceptibility to sclerotial formation was observed among five potato cultivars. In all cases, a large proportion of tubers showed superficial corky lesions, often associated with deformations. The proportion of tubers with lesions and deformations was highest in soil infested with AG 2-1 and significantly lower on cv. Samba in all treatments. All isolates were highly sensitive to flutolanil, iprodione and pencycuron, except the AG 5 isolates, moderately sensitive to pencycuron. These results show that, although AG 3 is the most common *R. solani* group on potato in France, AG 5 and AG 2-1 may be present. Isolates differed for pathogenicity. *In vitro* sensitivity to fungicides varied among AGs.

Abbreviations: AG – anastomosis group; EC_{50} – effective concentration causing 50% growth inhibition.

Introduction

Rhizoctonia solani Kühn (teleomorph: *Thanatephorus cucumeris* (Franck) Donk) is a soilborne pathogen responsible for severe damage on many crop species. On potato, this fungus causes delayed emergence, lesions on stems (stem canker) and stolons, and sclerotial formation on tubers (black scurf) (Baker, 1970; Anderson, 1982). *R. solani* attacks occur wherever potato is grown and may be responsible for yield reduction. They may also affect the quality of tubers through size distribution or alteration of skin aspect: sclerotia decrease tuber marketability. Attacks on stolons may induce the development of mis-shapen tubers (Weinhold et al., 1978; Escande and Echandi, 1991; Jeger et al., 1996).

Besides the typical stem canker and black scurf, other symptoms have also been occasionally attributed to *R. solani*, most notably scabby lesions on tuber skin which closely resemble netted scab symptoms caused by *Streptomyces reticuliscabiei* and some strains of *Streptomyces europaeiscabiei* (Bouchek-Mechiche et al., 2000), but also desquamation in restricted halos. While the implication of *R. solani* in these symptoms is not unequivocally established (Jouan, 1997; Turff, 2002), it is strongly suggested by the occurrence of such symptoms on cultivars resistant to netted scab and the possibility of observing and isolating *R. solani*

from diseased areas (B. Perraton, unpubl. data). The capacity of *R. solani* to cause such superficial lesions could be a manifestation of its saprophytic rather than pathogenic ability (Menzies, 1970). A better assessment of the aetiology of these superficial alterations is of upmost importance, as tuber aspect is a major quality factor for tubers sold washed for the fresh market.

R. solani is a collective species composed of groups classified following an anastomosis grouping system based on hyphal fusion. Thirteen anastomosis groups (AGs) have been identified, some of them being divided into sub-groups that differ for pathogenic, biochemical or genetic characteristics (Anderson, 1982; Cubeta and Vilgalys, 1997; Carling et al., 2002). AG 3 isolates are identified as the principal cause of *R. solani* attacks on potato (Anderson, 1982; Ogoshi, 1987; Carling and Leiner, 1990), but AG 1–AG 9 (mainly AG 4, AG 5 and AG 8) have been occasionally isolated from potato or from soils in which potato has been grown (Chand and Logan, 1983; Bandy et al., 1984; Carling and Leiner, 1990; Balali et al., 1995; Jeger et al., 1996; Bounou et al., 1999).

The most common method for protecting potato crops against *R. solani* is to use fungicides. A wide range of chemicals can be used, from the broad-spectrum dithiocarbamate metham sodium to the phenylurea pencycuron, which is extremely selective against *R. solani* and *Rhizoctonia*-like binucleate fungi (Jeger et al., 1996). However, no fungicide has been described as efficient against all species of *Rhizoctonia* (Kataria et al., 1991). Moreover, variation in sensitivity to fungicides has been repeatedly reported within *R. solani* (Martin et al., 1984; Sumner, 1987; Ueyama et al., 1990; Kataria et al., 1991; Csinos and Stephenson, 1999; Virgen-Calleros et al., 2000).

This paper describes the anastomosis groupings of 241 isolates of *R. solani* collected from potato plants with varying symptoms from different areas of France. Symptoms present on potato tubers of five different cultivars after artificial inoculation by three isolates belonging to different AGs are examined. The *in vitro* sensitivity of isolates to three fungicides of different chemical classes is reported.

Materials and methods

Fungal isolates

R. solani was isolated from potato tubers collected from various fields in different potato-growing areas in France. Isolates were cultured from stem canker or hymenia, sclerotia, superficial lesions or cavities on tubers, on malt ($20\,g\,l^{-1}$)–agar ($20\,g\,l^{-1}$) containing $250\,mg\,l^{-1}$ streptomycin.

For determination of AGs, standard isolates of *R. solani* AG 1 (01R01), AG 2-1 (21R21), AG 2-2 (22R01), AG 3 (3R41), AG 4 (04R02), AG 5 (05R01), AG 8 (0801) and AG 9 (1090) (Schneider et al., 1997b) were obtained from IPO-DLO (now Plant Research International, Wageningen, the Netherlands). One isolate from each AG, i.e. AG 3 (9729-1), AG 5 (9747) and AG 2-1 (9784A), was used for artificial inoculation. 9729-1 was obtained from stem canker on potato, 9747 and 9784A from superficial lesions with desquamation on tubers grown in different areas in France (Campion et al., 1999). All isolates were maintained on malt–agar at 20 °C.

Morphology and anastomosis grouping

Mycelium of all isolates visually relating to *R. solani* cultural morphology (white to brownish colour and sclerotial formation) (Parmeter and Whitney, 1970) was stained in $10\,g\,l^{-1}$ aniline blue, as described by Herr (1979). Mycelium was then examined under the microscope to check that each isolate had the mycelial characteristics of *R. solani* (multinucleate cells with septal pore apparatus) (Parmeter and Whitney, 1970). AGs were determined in petri dishes by pairing a plug (3 mm diameter plug taken from the margin of an actively growing colony on malt–agar) of each isolate with a plug of one *R. solani* standard isolate on water agar ($20\,g\,l^{-1}$). The petri dishes were then incubated at 20 °C until the growing hyphae from the opposite plugs overlapped. The overlapping portion was examined under the microscope for hyphal fusion as described by Parmeter et al. (1969). Isolates were considered to belong to the same AG if three or more fusions were observed. All isolates were first paired with the AG 3 standard isolate, and if no fusion occurred, with each of the seven other standard isolates.

Pathogenicity determination

Healthy stem cuttings of potato cvs. Bintje, Cynthia, Marine, Monalisa and Samba were produced *in vitro* on a medium containing (per litre): 536 mg NH_4NO_3, 472 mg $Ca(NO_3)_2 \cdot 4H_2O$, 419 mg $MgSO_4 \cdot 7H_2O$, 2690 mg KNO_3, 274 mg KH_2PO_4, 350 mg KCl, 27.85 mg $FeSO_4 \cdot 7H_2O$, 37.25 mg Na_2EDTA, 0.025 mg $CoCl_2 \cdot 6H_2O$, 0.025 mg $CuSO_4 \cdot 5H_2O$,

22.3 mg $MnSO_4 \cdot 4H_2O$, 0.83 mg KI, 0.25 mg $Na_2MoO_4 \cdot 2H_2O$, 8.6 mg $ZnSO_4 \cdot 7H_2O$, 6.2 mg H_3BO_3, 1 mg thiamin, 30 g sucrose, 5 g agar (pH adjusted to 6.0 before autoclaving). Inoculum of the three isolates 9729-1 (AG 3), 9747 (AG 5) and 9784A (AG 2-1) was produced by placing three plugs cut from the margin of cultures actively growing on malt–agar in bags containing 400 ml barley grains and 400 ml water that had been autoclaved twice for 1 h at 120 °C before inoculation. Mycelium was allowed to develop on the grains for 21 days at room temperature. For each isolate, six pots (1 l) were first filled with a 10 ml layer of infested grains (autoclaved barley for controls) mixed with potting mixture previously autoclaved at 120 °C for 30 min, and then filled to the top with disinfected potting mixture. One stem cutting was planted in each pot and placed in a greenhouse. Progeny tubers were harvested 11 weeks after planting and scored for the presence of superficial lesions, deformations and sclerotia. For each treatment, the total numbers of healthy tubers and of tubers exhibiting each symptom type were calculated by adding the results obtained in the six replicate pots. The same calculation was made for each cultivar (by adding results obtained for all treatments) and for each treatment (by adding results obtained for all cultivars). These data were compared using the likelihood ratio test (G-test) modified by Williams (1976) and Simultaneous Test Procedure according to Sokal and Rohlf (1981). Results were expressed as percentages of tubers exhibiting symptoms (by comparison with total number of tubers) for each treatment.

Fungicide sensitivity

R. solani isolates were assessed for fungicide sensitivity in petri dishes (90 mm diameter) containing 15 ml of fungicide-enriched malt–agar. The fungicide was added after autoclaving, resulting in the following concentrations: 0.01, 0.05, 0.1, 0.5 or 1 mg l^{-1} for flutolanil (benzanilide fungicide), 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5 or 10 mg l^{-1} for pencycuron (phenylurea fungicide) and 0.01, 0.05, 0.1, 0.5, 1 or 5 mg l^{-1} for iprodione (dicarboximide fungicide). Dishes without fungicide were also included as controls. Each dish was centrally inoculated with a 3 mm diameter mycelial plug taken from the margin of an actively growing *R. solani* colony on malt–agar. Three replicate dishes per isolate and fungicide concentration (including controls) were incubated at 20 °C. Colonies were measured along two diameters at right angles to one another 24 h after inoculation and when the mycelium reached the edge of the control dishes. Growth (total increase in diameter between two measurements) was calculated and the effective concentration causing 50% growth inhibition (EC_{50}) was determined from dose-relative inhibition curves using a log-probit scale, computer generated whenever possible.

Results

Anastomosis groups

Two hundred and twenty-six (94%) of the *R. solani* isolates tested anastomosed with the AG 3 standard isolate (3R41) (Table 1). They were mainly obtained from samples collected in Brittany (Finistère) and in Northern France (Pas-de-Calais and Somme). Among the 15 non-AG 3 isolates, five (2%) anastomosed with the AG 5 standard isolate (05R01) and 10 (4%) with the AG 2-1 standard isolate (21R21). AG 5 and AG 2-1 isolates were respectively recovered from only one geographical location, from the same field for all AG 5 isolates and from seven different fields in Finistère for AG 2-1 isolates (data not shown).

Most AG 3 and AG 2-1 isolates (respectively 185 out of 226 and seven out of 10) were obtained from sclerotia, whereas all AG 5 isolates were recovered from other types of symptoms (Table 2). Thirty-one AG 3, four AG 5 and one AG 2-1 isolates were collected from corky lesions. Some AG 3, AG 5 and AG 2-1 isolates were also obtained from scabby lesions or other superficial symptoms on tubers (cavities within deformations and desquamation in a restricted surface halo). Three AG 3 isolates were recovered from stem lesions or hymenia.

Table 1. AG of *R. solani* isolates collected from potato in France

Geographical origin	Number of isolates per AG		
	AG 3	AG 5	AG 2-1
Brittany			
Finistère	148	—	10
Morbihan	16	—	—
Côtes-d'Armor	3	—	—
Other origins			
Pas-de-Calais	43	—	—
Somme	10	—	—
Eure	3	—	—
Eure-et-Loir	1	—	—
Loiret	2	5	—
Total	226	5	10

Pathogenicity

Sclerotial formation on progeny tubers was observed only after inoculation with the 9729-1 (AG 3) isolate: 100% of tubers of cv. Bintje, around 90% of tubers of cvs. Cynthia, Marine and Monalisa and around 70% of tubers of cv. Samba showed sclerotia after 11 weeks growth in infested soil (Table 3). *G*-test and Simultaneous Test Procedure revealed no significant differences between cultivars for sclerotial formation on tubers.

Tuber skin alterations and/or tuber deformation were detectable in all treatments, including control plants (Figures 1 and 2). Infestation with the 9784A (AG 2-1) isolate caused significantly more superficial alterations than any other treatment, and cv. Marine was significantly less susceptible than the other four cultivars to this type of symptoms (Figure 1). For cvs. Marine and Monalisa, the proportion of tubers with superficial alterations did not differ statistically according to the inoculation treatment, while the remaining three cultivars showed different susceptibility to the various isolates used. For the three cultivars, the highest proportion of tubers with superficial alterations was observed with the AG 2-1 isolate.

More tubers showed deformations (Figure 2) than possessed superficial alterations. Deformation was highest in soil infested with the AG 2-1 isolate (Figure 1). The incidence of deformation was lower on

Table 2. Appearance of symptoms from which *R. solani* isolates were obtained

Symptom type	Number of isolates per AG		
	AG 3	AG 5	AG 2-1
Sclerotia	185	—	7
Corky lesions	31	4	1
Scabby lesions	3	1	1
Deformations	4	—	—
Desquamation	—	—	1
Stem lesions or hymenia	3	—	—

Table 3. Percentage of tubers showing sclerotia after artificial inoculation with *R. solani*

Cultivars	Percentages of tubers with sclerotia			
	Uninfested soil (a)[1]	AG 3 (b)[1]	AG 5 (a)[1]	AG 2-1 (a)[1]
Bintje	0 (a)[2]	100 (b)[2]	0 (a)[2]	0 (a)[2]
Cynthia	0 (a)[2]	86 (b)[2]	0 (a)[2]	0 (a)[2]
Marine	0 (a)[2]	91 (b)[2]	0 (a)[2]	0 (a)[2]
Monalisa	0 (a)[2]	89 (b)[2]	0 (a)[2]	0 (a)[2]
Samba	0 (a)[2]	72 (b)[2]	0 (a)[2]	0 (a)[2]

[1]Treatments followed by a common letter do not differ significantly according to the *G*-test and Simultaneous Test Procedure ($P < 0.001$).

[2]For each cultivar, values followed by a common letter do not differ significantly according to the *G*-test and Simultaneous Test Procedure ($0.001 < P < 0.01$).

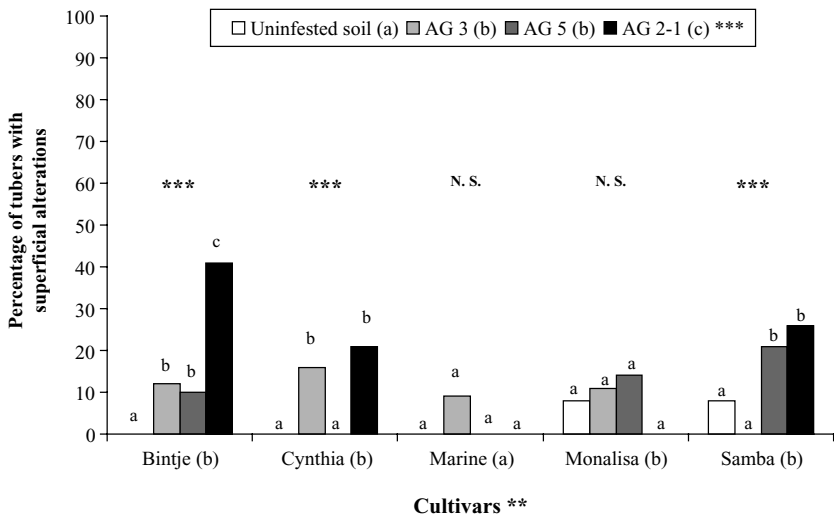

Figure 1. Percentage of tubers showing superficial alterations after artificial inoculation with *R. solani*. Cultivars (and for each cultivar, treatments) with a common letter do not differ significantly according to the *G*-test and Simultaneous Test Procedure. NS: not significant ($P > 0.05$); **: $0.001 < P < 0.01$; ***: $P < 0.001$.

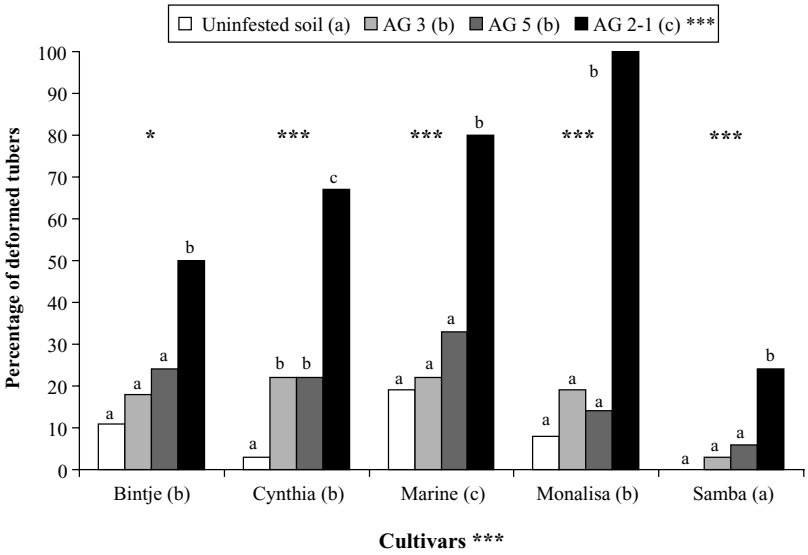

Figure 2. Percentage of tubers showing deformations after artificial inoculation with *R. solani*. Cultivars (and for each cultivar, treatments) with a common letter do not differ significantly according to the *G*-test and Simultaneous Test Procedure. *: $0.01 < P < 0.05$; ***: $P < 0.001$.

cv. Samba and higher on cv. Marine than on the three other cultivars. Corky lesions were frequently associated with deformation but symptoms similar to those from which 9747 and 9784A isolates were obtained were never observed on progeny tubers. *R. solani* isolates could be recovered only from sclerotia, they all had AG 3 characteristics.

Fungicide sensitivity

All *R. solani* isolates tested were highly sensitive to flutolanil, with EC_{50} values ranging between 0.05 and 0.50 mg l^{-1} (Figure 3). A larger variability occurred with pencycuron, where AG 3 and AG 2-1 isolates exhibited extreme sensitivity ($EC_{50} < 30\,\mu g\,l^{-1}$) whereas AG 5 isolates were only moderately sensitive to this fungicide (EC_{50} 1–5 mg l^{-1}) (Figure 4). All isolates were highly sensitive to iprodione ($EC_{50} < 1$ mg l^{-1}), except for two AG 2-1 isolates (9729-123 and 9829-12) with $EC_{50} > 2$ mg l^{-1} (Figure 5).

Discussion

Our results show that AG 3 is the predominant group of *R. solani* recovered from potato in France; this AG was isolated in all sites sampled. However, AG 5 and AG 2-1 were also found. These results confirmed previous observations: high representation of AG 3 and the presence of AG 5 (observed on Leguminosae, Ogoshi, 1987) as a small proportion of isolates on potato (Chand and Logan, 1983; Bandy et al., 1984; Balali et al., 1995; Jeger et al., 1996). AG 2-1 has already been reported from potato (Chand and Logan, 1983; Carling and Leiner, 1986), but less commonly than AG 5, and this, to our knowledge, is the first report of the isolation of AG 2-1 from potato plants in France.

AG 2-1 and AG 5 isolates were associated with geographically well-distinct locations. These two groups could be present in a greater number of sites, but not easily recovered because of fungus poor survival on tubers, stored after harvest. Indeed, all the AG 5 isolates were collected from freshly harvested tubers. Furthermore, we were not able to re-isolate the pathogen from symptomatic plants harvested from soil infested with either AG 5 or AG 2-1. Their presence may also be related to the previous crops cultivated in the fields, as observed in Southern Australia by Balali et al. (1995). AG 2-1 and AG 5 cannot be described as common in France, but they are not inconsequential and it would certainly be useful to determine precisely the distribution of the rare AGs in France, on freshly harvested tubers in a large number of sites.

AG 2-1 is a sub-group of AG 2, present on many crops, though mainly pathogenic on Cruciferae

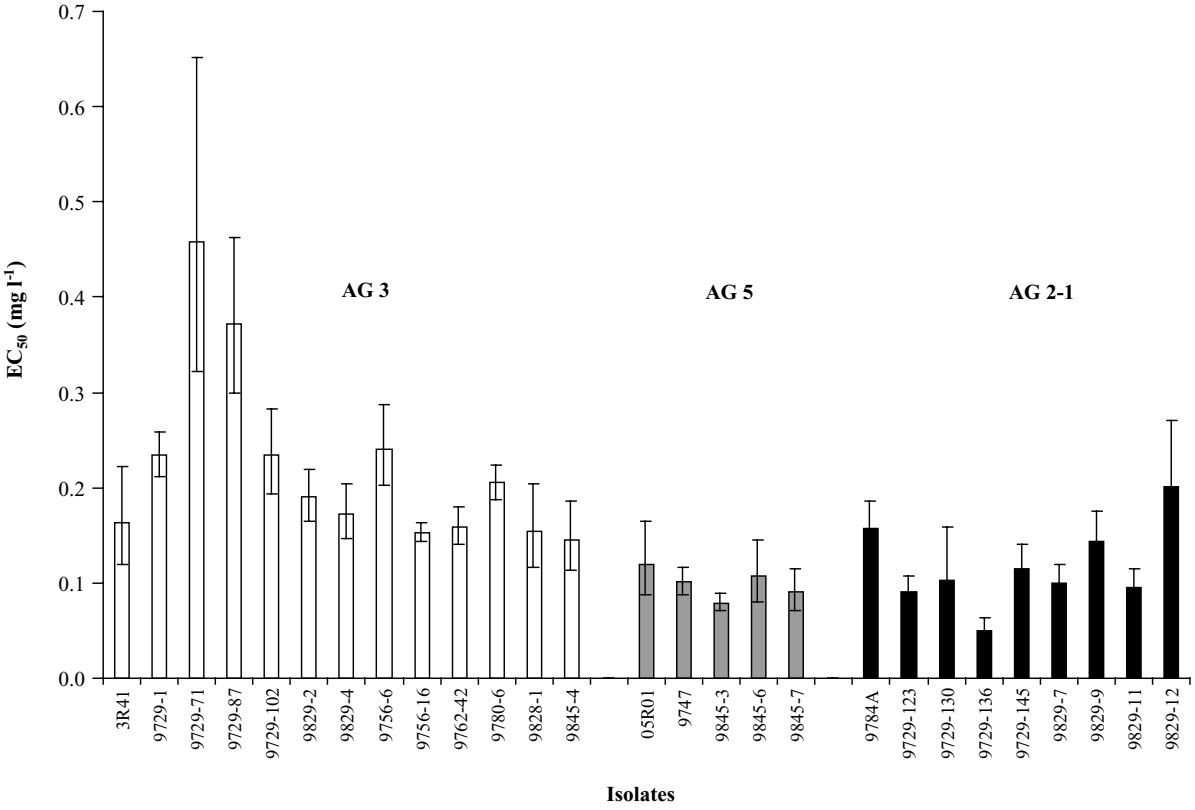

Figure 3. Sensitivity of *R. solani* isolates to flutolanil. Shown are mean EC_{50} values and their standard deviations.

(Anderson, 1982). AG 2-1 is a heterogenous group (Vilgalys and Gonzalez, 1990; Carling et al., 2002), in which some isolates (AG 2-t) are also pathogenic on Liliaceae (Schneider et al., 1997a). As our isolates anastomosed with the AG 2-1 standard isolate provided by IPO-DLO, we suggest that they are strictly AG 2-1. Nevertheless, as hyphal fusion may happen between AG 2-1 and AG 2-t (Schneider et al., 1997a), it would be interesting to further characterize these AG 2-1 isolates, for example with the molecular techniques described by Schneider et al. (1997b) and Salazar et al. (2000).

AG 3 and AG 2-1 collected in this study came mainly from sclerotia, the survival form of *R. solani*, but all AG 5, some AG 3 and AG 2-1 isolates were obtained from symptoms without sclerotia: corky or scabby lesions, cavities on deformed tubers and restricted surface halo with desquamation. It is difficult to determine whether *R. solani* was the causal agent of these symptoms, because saprophytic mycelium is able to remain viable on the tuber surface, for example, in cavities around eyes or within lenticels and periderm (Leach and Garber, 1970).

The three isolates used for artificial inoculation exhibited variability in pathogenicity on tubers. As they were from different AGs, these differences might reflect pathogenicity differences either between AGs (Balali et al., 1995) or within AGs (Carling et al., 2002). Artificial inoculation with several isolates per AG would be needed to decide between these two hypotheses.

After artificial inoculation with *R. solani,* superficial alterations (corky lesions) were observed on tubers, whereas restricted surface haloes with desquamation (similar to those from which the 9747 and 9784A isolates were obtained) were not observed. As a large part of isolates obtained in this study came from corky lesions, the observation of these symptoms after artificial inoculation reinforces the possibility that *R. solani* is directly or indirectly responsible for these symptoms, although their observation in control pots suggests that these alterations could have multiple origins, including physiological reactions.

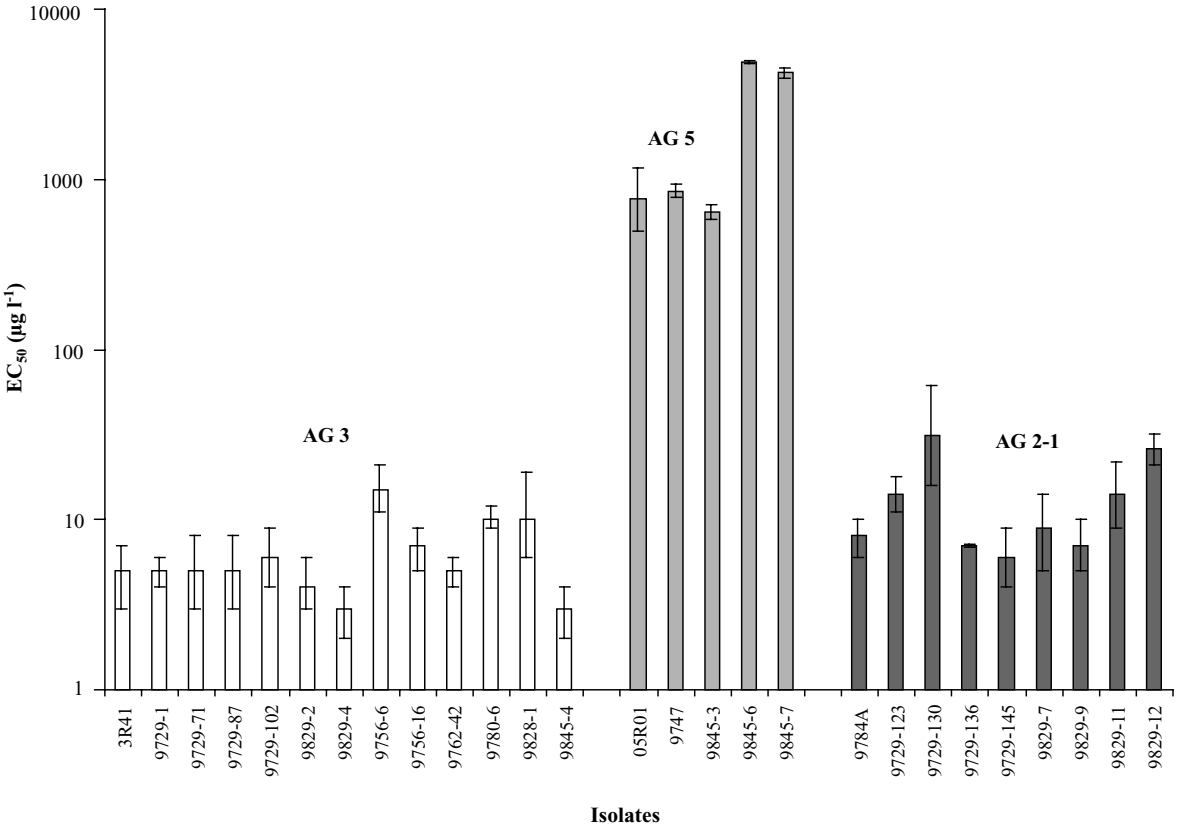

Figure 4. Sensitivity of *R. solani* isolates to pencycuron. Shown are mean EC_{50} values on a logarithmic scale, and their standard deviations.

Corky lesions can be associated with tuber deformations, considered to be the result of severe *R. solani* attacks on stolons (Scholte, 1989; Jeger et al., 1996). Percentages of deformed tubers after treatment with the AG 3 isolate were in agreement with previous results (Escande and Echandi, 1991) but this is, to our knowledge, the first report of implication of an AG 2-1 isolate in deformation of potato tubers. Moreover, though AG 2-1 is able to induce stem canker and sclerotial formation on tubers (Chand and Logan, 1983), it is generally considered to be an inconsequential or occasional pathogen for potato (Carling et al., 1989; Schneider et al., 1997a). Confirmation of the pathogenic ability of AG 2-1 would need successful re-isolation. This could be more easily obtained by sampling on stolons before or during tuberization than by sampling on mature tubers.

The AG 5 isolate used in this study was poorly aggressive on tubers. This might be a characteristic of the particular isolate used rather than of the AG, because AG 5 isolates were previously shown to cause symptoms on potato stems and tubers (Balali et al., 1995). In the same way, Bandy et al. (1984) mentioned pathogenicity differences within a group of nine AG 5 isolates collected in a potato field in Maine (USA). Our experimental conditions may have been unfavourable for expression of aggressiveness by AG 5, which generally thrives at temperatures of 15–20 °C (Carling and Leiner, 1990), and for survival of this isolate, since re-isolation from symptoms on tubers grown in inoculated soil was unsuccessful.

In our conditions, only the AG 3 isolate allowed us to reproduce sclerotial formation on tubers. As this phenomenon is induced by senescence (Anderson, 1982), a longer delay between vine destruction and harvest could have been more favourable to sclerotial formation with AG 5 and AG 2-1 isolates (Chand and Logan, 1983; Balali et al., 1995).

Potato cultivars exhibited no significant difference for susceptibility to sclerotial formation. The lower susceptibility of cv. Marine to superficial alteration and its higher susceptibility to deformation can confirm

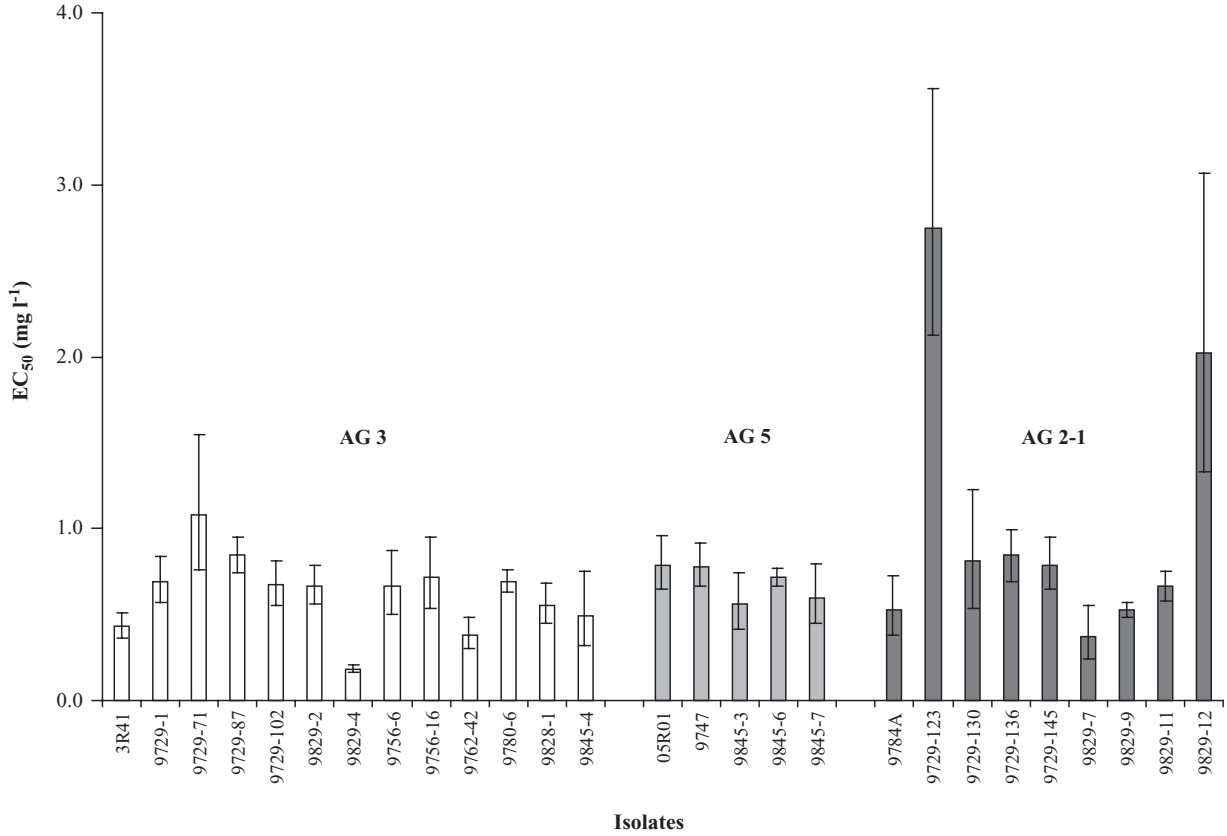

Figure 5. Sensitivity of *R. solani* isolates to iprodione. Shown are mean EC_{50} values and their standard deviations.

the implication of different AGs, AG 3 and AG 2-1 in the case, inducing two host–parasite relationships leading to the complex symptomatology (different types of tuber skin alteration and/or tuber deformation) due to *R. solani*. Cultivar Samba was less susceptible to deformation (associated with superficial alterations) by comparison with other cultivars, but these results should be complemented with field data.

Following the classification given by Martin et al. (1984), the three fungicides tested were highly active against *R. solani* isolates. EC_{50} values to iprodione and flutolanil were of the same order as the ones previously mentioned for *R. solani* (Martin et al., 1984; Csinos and Stephenson, 1999). Efficiency of pencycuron against the AG 5 isolates was moderate, confirming previous data (Kataria and Verma, 1989). According to Virgen-Calleros et al. (2000), differences in sensitivity to pencycuron, a specific fungicide developed for use against *Rhizoctonia* sp. on rice and potato (Ueyama et al., 1990), would depend on the capacity of isolates to metabolize the active ingredient. Further work should be undertaken about the direct effects of fungicides on *R. solani* isolates.

This work has provided a better knowledge about *R. solani* groups found on potato in different locations in France. It has confirmed the predominance of AG 3 isolates, but it has also shown the presence of AG 5 and AG 2-1. Various symptoms were observed on progeny tubers after artificial inoculation with isolates from different AGs. This part of the study should be continued with several isolates per AG, to determine the origin of the pathogenicity variations, and to confirm that AG 2-1 isolates may be pathogenic on potato tubers. Further work should be undertaken to evaluate the fungicides used in this study for efficiency in controlling *R. solani* attacks on potato in greenhouse or field trials and to confirm differences among AGs in sensitivity to one fungicide. This study has shown the importance of characterization of *R. solani* isolates present on potato for production of high quality tubers.

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