Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/sjar

Spanish Journal of Agricultural Research 2011 9(1), 303-307
ISSN: 1695-971-X
eISSN: 2171-9292

Short communication. The wilt of winter cucumber in south-eastern Spain caused by *Pythium irregulare*

J. M. Gomez-Vazquez[1]*, Y. Serrano-Alonso[1] and J. M. Melero-Vara[2]

[1] *Instituto de Investigación y Formación Agraria y Pesquera (IFAPA). Centro La Mojonera. Camino de San Nicolás, 1. 04745 La Mojonera (Almería). Spain*
[2] *Instituto de Agricultura Sostenible. CSIC. Alameda del Obispo, s/n. 14004 Córdoba. Spain*

Abstract

Pythium irregulare was isolated from adult cucumber plants with stem base necrosis, wilt or, frequently, that were dead; symptoms were particularly observed in crops grown in winter in plastic-houses in south eastern Spain. Damage was often important because symptoms develop during the fruit harvesting period. Cucumber plants cv. Nevada sown in late September were inoculated at the 4-7 leaf stage (mid October) with a suspension of propagules of *P. irregulare* isolated from diseased cucumber or tomato plants. Non-inoculated plants served as controls. Symptoms were recorded weekly until the end of the experiment in mid January and cumulative marketable yields calculated. The experiment was repeated the following year. A high incidence (93.1 and 97.9%) of diseased or dead plants occurred in plots inoculated with isolates of *P. irregulare* from cucumber, which were significantly more aggressive than tomato isolates. Plants in non-inoculated plots remained symptom free. Yields were significantly lower in plots inoculated with cucumber isolates in both years, mainly due to reductions in the number of fruits rather than fruit weight. This is the first report of *P. irregulare* as the causal agent of root rot in plastic-houses of cucumber plants in Almería (Spain), one of the world's largest concentrations of plastic-houses.

Additional key words: aetiology; *Cucumis sativus;* fruit yield; oomycetes; soil-less crops; zoosporic pathogens.

Resumen

Comunicación corta. La marchitez del pepino invernal causada por *Pythium irregulare* en el sureste de España

Pythium irregulare se aisló de plantas adultas de pepino con necrosis en la base del tallo, marchitez y, a menudo, muertas; síntomas que fueron particularmente observados en los cultivos de invierno de los invernaderos del sudeste de España. Los daños fueron frecuentemente importantes porque los síntomas se manifestaron durante la recolección de los frutos. A mediados de octubre se inocularon plantas de pepino del cv. Nevada, en estado de 4-7 hojas verdaderas, que habían sido sembradas a finales de septiembre. La inoculación se realizó con suspensiones acuosas de estructuras de varios aislados de *P. irregulare* obtenidos de plantas enfermas de pepino o tomate. Plantas no inoculadas sirvieron como testigos. Los síntomas se anotaron semanalmente hasta que finalizó el experimento a mediados de enero, y se calculó la producción acumulada de frutos comercializables. El experimento se repitió el año siguiente. Se observó un alto porcentaje (entre el 93,1 y el 97,9%) de plantas marchitas o muertas, en las parcelas inoculadas con los aislados de *P. irregulare* obtenidos de pepino, que fueron más agresivos que los de tomate. Las plantas no inoculadas permanecieron sin síntomas. En ambos experimentos, la producción de frutos fue significativamente más baja en las parcelas inoculadas con los aislados obtenidos de pepino, debido principalmente a la reducción del número de frutos más que al peso de éstos. Se señala por vez primera a *P. irregulare* como agente causal de la podredumbre de raíces de pepino en invernaderos de Almería (España), con la mayor concentración de invernaderos de plástico del mundo.

Palabras clave adicionales: *Cucumis sativus;* cultivo sin suelo; etiología; mermas de cosecha; oomycetos; patógenos zoospóricos.

Intensive horticulture has much developed along the southern Mediterranean coast of Spain during the past 30 years and there are now an estimated 27,000 ha of plastic-houses in Almería and Granada provinces (Sanjuán, 2004). To prevent diseases caused by soil-borne pathogens there has been increasing interest in the use of open hydroponics systems for vegetable production during the last 15 years with an estimated 5,000 ha in plastic-houses in 2006 (Céspedes *et al.*,

* Corresponding author: juliom.gomez@juntadeandalucia.es
Received: 26-02-10; Accepted: 16-12-10.

2009). The substrates most commonly used in hydroponics are perlite bags and rockwool slabs. These crop systems were originally intended mainly to avoid root diseases (Zinnen, 1988; Stanghellini and Rasmussen, 1994a). However, elsewhere in the world and also here, soil-less systems also provide optimal conditions for zoosporic pathogens to spread and multiply rapidly (Stanghellini and Rasmussen, 1994a,b; Gómez, 2000) because *Pythium* spp. are well adapted to aqueous media (Hendrix and Campbell, 1973; Blancard *et al.*, 1992; Martin and Loper, 1999). Long cucumber (*Cucumis sativus*) is widely cropped in south Mediterranean coastal provinces of Spain with 7,350 ha in 2008 (Anonymous, 2009), with ca. 1,100 ha being cultivated in soil-less systems (García, personal communication).

Many *Pythium* spp. may cause damping-off of seedling in vegetables and may kill feeder roots but are seldom able to kill mature plants (Hendrix and Campbell, 1973). Cucumber seedlings are susceptible to *Pythium aphanidermatum*, *P. ultimum*, *P. myriotylum*, *P. debaryanum*, *P. irregulare*, *P. sylvaticum*, *P. coloratum* and isolates belonging to *Pythium* «group G». Symptoms usually comprise reduced growth, stem base and root necrosis and pre-emergence damping-off, with damage in artificial inoculation ranging from slight to complete plants loss, depending on the fungal species, the aggressiveness of isolates and environmental conditions (Jenkins and Averre, 1983; Favrin *et al.*, 1988; Blancard *et al.*, 1992; Moulin *et al.*, 1994; Messiaen *et al.*, 1995; Martin and Loper, 1999; Herrero *et al.*, 2003). Specifically, *P. irregulare* caused damping-off at 20 and 24°C but not at 28 and 32°C (Ben-Yephet and Nelson, 1999).

Meanwhile, adult cucumber plants are susceptible to *P. aphanidermatum,* causing severe damage at temperature above 25°C, and *P. ultimum* is more aggressive at temperatures below 15°C, *i.e.* in non-heated greenhouses during winter. Others species, such as *P. irregulare* cause root necrosis and wilting during the hot hours of sunny days since the beginning of fruit harvesting until the end of the crop cycle, which appears to determine low yields (Favrin *et al.*, 1988; Blancard *et al.*, 1992).

P. aphanidermatum seems to be, for the commercial cucumber production, the most important pathogenic species of *Pythium* in different crop areas (Jenkins and Averre, 1983; Gold and Stanghellini, 1985; Favrin *et al.*, 1988; Blancard *et al.*, 1992; Messiaen *et al.*, 1995), including the plastic-houses of the southern Mediterranean coast of Spain. In this area, dead cucumber plants occurring at any growth stage are frequently found, especially when they are grown in non-disinfested soil or in re-used substrates. *P. aphanidermatum* is prevalent during August-October and March-June for autumn-sown and spring-sown crops, respectively (Gómez, 2003). This work aimed to determine the aetiology of the disease that causes cucumber wilt and death during November to February in that area.

Two experiments were conducted in a plastic-house located at Campo de Dalías (36.76-36.81 N, 2.58-2.92 W), Almería, Spain; the plastic-house was covered with 200-μm thick transparent low density polyethylene film (Sotrafa, Almería, Spain). Pre-germinated seeds of cucumber cv. Nevada were sown directly in perlite bags in late September. Irrigation water had an electric conductivity of 0.5 to 0.6 dS m^{-1}; a nutrient solution of 1.9 to 2.1 dS m^{-1} was prepared in a 25 m^3 tank for drip irrigation, with drainage of about 20% of the water volume used. Cucumber plants at the 4-7-leaf stage were inoculated with one of two mixtures each containing two isolates of *P. irregulare, i.e.,* Py-14 plus Py-20 or Py-52 plus Py-53, obtained from diseased cucumber and tomato plants respectively. These isolates were identified on the basis of morphology of sporangia and oogonia (Van der Plaats-Niterink, 1981) and selected by the high mortality caused to cucumber seedlings and plants in winter (Gómez, 2003). For the two inoculation experiments conducted in greenhouse, the contents of two 90-mm diameter Petri dishes with potato-carrot-agar fully covered by colonies of the two isolates of each mixture were blended and homogenized with 500 mL distilled water to obtain concentrations of 1-5 10^2 colony-forming units mL^{-1}. Inocula were poured around cucumber plants at a rate of 50 mL $plant^{-1}$ in mid-October. The experimental design was a complete randomised block with four replicates with individual plots comprising 18 plants (3 plants per perlite bag). Symptoms, mainly wilt, stem base necrosis and plant death were recorded weekly along the experimental periods. Weights and numbers of marketable fruits were recorded sequentially for the different plots from mid October to mid January and cumulative curves of cucumber fruit production calculated. Data of these variables for different inocula were analysed by ANOVA for each experiment (significance levels indicated as *p*-values in Table 1) and mean values were compared using Fisher's protected least significant difference (LSD) test at $\alpha < 0.05$ significance level.

In experiment 1, the first symptoms of the disease, involving wilting of plants, were observed 45 days after inoculation with the mixture of *P. irregulare* isolates obtained from cucumber plants; plants inoculated with

Table 1. Final incidence of diseased and dead plants and cumulative marketable yield of cucumber cv. Nevada inoculated with a mixture of two *Pythium irregulare* isolates from two host plants

Source of isolate	Symptomatic plants[1] (%)	Dead plants[1] (%)	Marketable yield[1] (kg plant^{-1})	Number[1] (marketable fruit plants^{-1})	Fruit weight (g fruit^{-1})
Experiment 1					
F[2]	94.6*	21.1*	8.6*	8.7*	1.15
Control	0.0^{b}	0.0^{c}	4.9^{a}	13.2^{a}	371
Cucumber	97.9^{a}	97.9^{a}	4.1^{b}	10.6^{b}	385
Tomato	75.0^{a}	52.1^{b}	4.7^{a}	12.3^{a}	381
Experiment 2					
F[2]	87.4*	168.1*	25.7*	22.2*	0.09
Control	0.0^{c}	0.0^{b}	6.1^{a}	15.5^{a}	394
Cucumber	93.1^{a}	66.7^{a}	3.8^{b}	9.7^{b}	392
Tomato	13.9^{b}	2.8^{b}	5.5^{a}	14.0^{a}	393

[1] In each column, values followed by the same letter are not statistically different ($p < 0.05$) according to Fisher's protected LSD test.[2] *F* values of the ANOVA. * Significant differences ($p < 0.05$).

isolates from tomato developed symptoms a few days later (Fig. 1). Stem base necrosis was infrequent. Significantly higher ($p < 0.05$) incidences of diseased and dead plants were found when inoculation was with *P. irregulare* isolates from cucumber plants, whereas plants inoculated with those from tomato plants showed lower disease incidences. Non-inoculated plots remained disease free.

From day 26 to day 76 after planting the incidence of wilted plants rapidly increased to ca. 81% with *P. irregulare* isolates from cucumber and, simultaneously, to 68% in plants inoculated with isolates from tomato. Plant death first occurred when the maximum incidence of wilted plants was reached, and then rose sharply to 97% and 52% in plants inoculated with cucumber and tomato isolates respectively (Table 1). All inoculated plant showed symptoms of necrotic lesions on the roots.

Cumulative marketable yields showed significant ($p < 0.05$) reductions in the last 2-3 wk of the experiment in plants inoculated with *P. irregulare* isolates

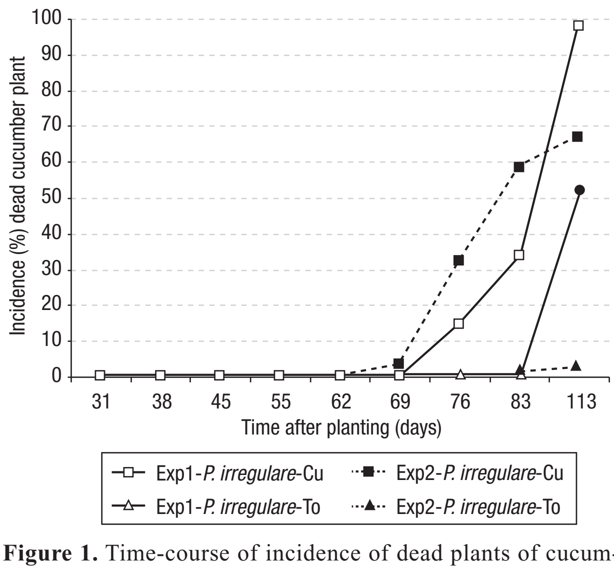

Figure 1. Time-course of incidence of dead plants of cucumber cv. Nevada inoculated with a mixture of two isolates of *Phytium irregulare* obtained from diseased plants of cucumber (Cu) or tomato (To) (experiments 1 and 2).

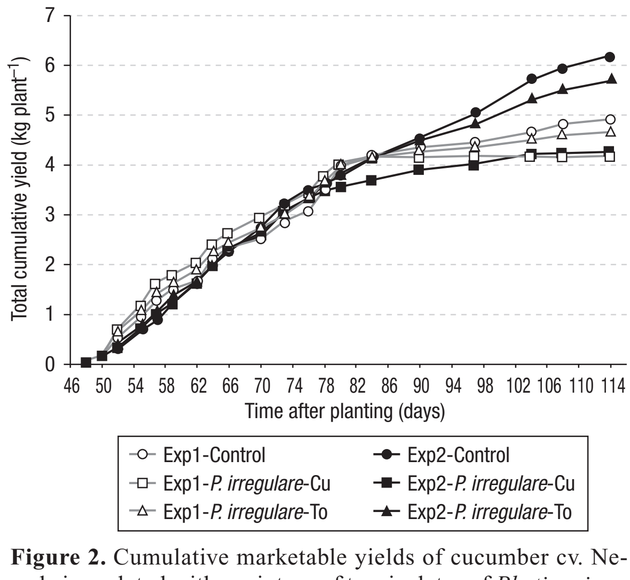

Figure 2. Cumulative marketable yields of cucumber cv. Nevada inoculated with a mixture of two isolates of *Phytium irregulare* obtained from diseased plants of cucumber (Cu) or tomato (To) (experiments 1 and 2).

from cucumber (Fig. 2). In contrast, cucumber yields did not differ between plots inoculated with *P. irregulare* isolates obtained from diseased tomato plants and non-inoculated (control) plots (Table 1). The final yield reduction for plants inoculated with *P. irregulare* isolates from cucumber plants averaged 16.3%, and was significantly greater ($p < 0.05$) in comparison with non-inoculated plants; this reduction was related to the number of fruits harvested and not to their weight (Table 1). In contrast, yield reductions in plots inoculated with *P. irregulare* isolated from tomato averaged only 4.1% and did not differ ($p < 0.05$) from the control plots (Fig. 2).

The development of the disease in experiment 2 followed a similar trend, although rates of wilted and dead plants were lower than those obtained in experiment 1 (Table 1; Fig. 1). The final marketable yield reduction for plants inoculated with *P. irregulare* isolates from cucumber plants averaged 36.7%, and was significantly greater ($p < 0.05$) in comparison with non-inoculated plants. Yield reductions in plots inoculated with *P. irregulare* isolated from tomato averaged 8.3% and did not differ ($p < 0.05$) from the control plots (Table 1). The pathogen was consistently recovered from symptomatic plants in both experiments and a significant reduction in the number of marketable fruits produced was observed when the plants were inoculated with the mixture of *P. irregulare* isolates recovered from diseased cucumber plants only, but fruit weight was not affected in any case (Table 1).

The pathogenicity of *P. irregulare* to adult cucumber plants, associated with the coldest months of the year, was demonstrated in both experiments by the frequent wilting, occasional stem base necrosis, and plant death. Symptoms did not appear until mid December when environmental conditions became favourable, but thereafter the epidemics developed rapidly. Also, yield reduction caused by *P. irregulare* during the months of the year when the fruit harvested reach a higher market value was demonstrated. The higher disease incidence observed in our experiments with the two cucumber isolates in comparison with tomato isolates may suggest a degree of isolate host specificity, but this requires verification with larger numbers of isolates from the two hosts.

The results differed from those of Moulin *et al.* (1994) who produced damping-off in cucumber seedlings inoculated with *P. irregulare* but failed to produce symptoms in adult cucumber plants; however they reported symptoms when adult cucumber plants were inoculated with *P. aphanidermatum,* probably because their incubation temperature was inadequate for *P. irregulare* infection. Disease caused by *P. irregulare* has been reported elsewhere *e.g.* in France, but only slight symptoms of root necrosis were reported (Blancard *et al*., 1992). However, our results are more in agreement with those obtained by Favrin *et al.* (1988), when cucumber plants were inoculated with *P. irregulare* and then incubated in a growth chamber, although the plants growth and environmental conditions in their experiments were completely different.

Under the winter conditions of SE Spain, the symptoms caused by *P. irregulare* on cucumber differ from those caused by *P. aphanidermatum*, with the latter species giving rise to severe damage at high temperatures (35°C) but causing only a moderate incidence of plant death and yield loss in autumn-sown crops (Gómez, 2003). In contrast, a high incidence of dead plants, but a lower incidence of stem base necrosis and relatively moderate yield production occurred with *P. irregulare* in winter (minimum and maximum temperatures ranging 8-10°C and 25-28°C, respectively). The low incidence of stem base necrosis observed in cucumber plants inoculated with *P. irregulare* seems to be due mainly to the age of the plants at inoculation rather than to the pathogen itself, and similar observations were made with *P. aphanidermatum* (Gómez, 2003). Future studies are needed to assess the distribution and control of the disease in winter cucumber crops grown in soil and in soil-less culture systems in plastic-houses of SE Spain.

Acknowledgments

We are grateful to Josefina Ros for her technical help and the *Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria* (INIA) for financial support.

References

ANONYMOUS, 2009. Estadísticas agrarias. Consejería de Agricultura y Pesca de la Junta de Andalucía, Sevilla, Spain. [In Spanish].

BEN-YEPHET Y., NELSON E.B., 1999. Differential suppression of damping-off caused by *Pythium aphanidermatum*, *P. irregulare* and *P. myriotylum* in composts at different temperatures. Plant Dis 83, 356-360.

BLANCARD D., RAFIN C., CHAMONT S., TIRILLY Y., JAILLOUX F., 1992. Phénomène de perte de racines en

culture hors sol. Rôle des *Pythium* spp. PHM Revue Horticole 329, 35-45. [In French].
CÉSPEDES A.J., GARCÍA M.C., PÉREZ J.J., CUADRADO I.M., 2009. Caracterización de la explotación hortícola protegida de Almería (Cuadrado I.M., ed). FIAPA, Almería, Spain. 177 pp. [In Spanish].
FAVRIN R.J., RAHE J.E., MAUZA B., 1988. *Pythium* spp. associated with crown rot of cucumbers in British Columbia greenhouses. Plant Dis 72, 683-687.
GOLD S.E., STANGHELLINI M.E., 1985. Effect of temperature on *Pythium* root rot of spinach grown under hydroponic conditions. Phytopathology 75, 333-337.
GÓMEZ J., 2000. Consideraciones sobre la sanidad de los cultivos hortícolas sobre sustratos. Actas de Horticultura. V Jornadas de Sustratos de la Sociedad Española de Ciencias Hortícolas, Almería, Noviembre. pp. 191-207. [In Spanish].
GÓMEZ J., 2003. Enfermedades del melón y pepino en los cultivos sin suelo del sudeste andaluz. PhD thesis, University of Almería. 253 pp. [In Spanish].
HENDRIX F.F., CAMPBELL W.A., 1973. *Pythium* as plant pathogens. Annu Rev Phytopathol 11, 78-98.
HERRERO M.L., HERMANSEN A., ELEN O.N., 2003. Occurrence of *Pythium* spp. and *Phytophthora* spp. in Norwegian greenhouses and their pathogenicity on cucurber seedlings. J Phytopathol 151, 36-41.
JENKINS Jr. S.F., AVERRE C.W., 1983. Root diseases of vegetables in hydroponic culture systems in North Carolina greenhouses. Plant Dis 67, 968-970.
MARTIN F.M., LOPER J.E., 1999. Soilborne plant diseases caused by *Pythium* spp.: ecology, epidemiology, and prospects for biological control. Crit Rev Plant Sci 18, 111-181.
MESSIAEN C.M., BLANCARD D., ROUXEL F., LAFON R., 1995. Enfermedades de las hortalizas. Ed Mundi-Prensa, Madrid. 576 pp. [In Spanish].
MOULIN F., LEMANCEAU P., ALABOUVETTE C., 1994. Pathogenicity of Pythium species on cucumber in peat-sand rockwool and hydroponics. Eur J Plant Pathol 100, 3-17.
SANJUÁN J.F., 2004. Estudio multitemporal sobre la evolución de la superficie invernada en la provincia de Almería por términos municipales desde 1984 hasta 2004, mediante teledetección de imágenes Thematic Mapper de los satélites Landsat V y VII (Cuadrado I.M., ed). FIAPA, Almería, Spain. 98 pp. [In Spanish].
STANGHELLINI M.E., RASMUSSEN S.L., 1994a. Identification on origin of plant pathogenic microorganisms in recirculating nutrient solutions. Adv Space Res 14, 349-355.
STANGHELLINI M.E., RASMUSSEN S.L., 1994b. Hydroponics. A solution for zoosporic pathogens. Plant Dis 78, 1129-1138.
VAN DER PLAATS-NITERINK A.J., 1981. Monograph of genus *Pythium*. Studies of Mycology 21. Centraalbureau voor Schimmelcultures, Baarn, NL. 242 pp.
ZINNEN T.M., 1988. Assessment of plant diseases in hydroponic culture. Plant Dis 72, 96-99.