THE ECONOMIC IMPACT OF THE BACTERIAL BLIGHT OF SOYBEAN UNDER EUROPEAN AGROCLIMATIC CONDITIONS

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SUMMARY

The economic impact of the bacterial blight of soybean caused by *Pseudomonas syringae* pv. *glycinea* has been investigated in three countries belonging to the European Union: Italy, France and Spain. Weather and growing conditions have been monitored over three years of field experiments (1992-1994) and the data analysed in order to evaluate possible yield losses and in view of the production of pathogen free seed. In Italy and France, using different cultivars and seed with a contamination level of 0.5-20% no significant yield losses were found. In Italy the initial seed contamination level was positively correlated with the contamination of the harvested seed by the pathogen; both in Italy and in France on some cultivars, it was possible to correlate seed contamination level with the epiphytic population of the pathogen and the intensity of symptoms affecting plants in the field. No epiphytic contamination by the pathogen was observed in Spain, even at the highest seed contamination rate (20%), and there was no disease in the field and no yield reduction. The pathogen seemed not to become systemic since no contamination was observed on seed aseptically taken in the field just before harvest. The experiments highlighted the low impact of soybean bacterial blight under European climatic conditions, but suggested the choice of dry and warm regions for the production of quality seed to prevent the accumulation of effective inoculum on the seed year by year.

RIASSUNTO

L'IMPATTO ECONOMICO DELLA MACULATURA BATTERICA DELLA SOIA NELLE CONDIZIONI AGROCLIMATICHE EUROPEE. In tre Stati Membri dell'Unione Europea, Italia, Francia e Spagna, è stato valutato l'impatto economico dell'avvizzimento batterico della soia causato da *Pseudomonas syringae* pv. *glycinea*. Nelle aree in cui erano stati approntati i campi sperimentali, durante il triennio 1992-94, sono stati monitorati i dati climatici, agronomici e colturali, utilizzati poi per valutare le perdite del prodotto provocate dalla malattia e verificare la possibilità di produrre seme esente dal patogeno. In Italia ed in Francia, usando cultivars differenti di soia il cui seme era stato sperimentalmente contaminato con livelli di contaminazione che andavano dallo 0,5 al 20%, non ci sono state perdite statisticamente significative di prodotto alla raccolta. In Italia, però, i differenti livelli di contaminazione del seme di partenza sono stati positivamente correlati alla contaminazione del seme raccolto. Per alcune varietà coltivate in Italia ed in Francia è stata osservata anche una correlazione positiva tra contaminazione del seme di partenza, livello della popolazione epifita del patogeno durante la stagione vegetativa ed intensità dei sintomi sulle piante in campo. Nei campi sperimentali spagnoli non è mai stata rilevata alcuna contaminazione epifita, come pure non è mai stato osservato alcun sintomo della malattia o significative riduzioni del seme raccolto, anche quando il 20% del seme di partenza era stato sperimentalmente contaminato. Non ci sono state evidenze sperimentali che la malattia possa diventare sistemica poiché nessuna contaminazione è mai stata rilevata nel seme prelevato asetticamente in campo prima della trebbiatura. La sperimentazione effettuata ha messo in luce il basso impatto agromomico dell'avvizzimento batterico della soia in differenti aree europee, ma suggerisce la coltivazione di questa leguminosa in aree con clima caldo ed asciutto nel caso si voglia produrre seme di qualità.

Key words: bacterial blight, European Union, *Pseudomonas syringae* pv. *glycinea*, soybean.

INTRODUCTION

Bacterial blight of soybean caused by *Pseudomonas syringae* pv. *glycinea* (Psg) occurs world-wide in the major growing areas (Kennedy and Tachibana, 1973). In the United States this disease does not normally affect

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yield, as it occurs in the early growing stages and growth compensates the loss of photosynthetic area (Teigen and Vorst, 1975). However, bacterial blight may cause substantial losses if susceptible cultivars are grown under unfavourable conditions (Williams and Nyvall, 1980; Park and Lim, 1985); among procaryotes, Psg caused the greatest dollar-loss per year in the most important soybean growing state Iowa in 1976 (Kennedy and Alcorn, 1980). The pathogen is seed transmitted over long distances. In the member states of the European and Mediterranean Plant Protection Organization (the EPPO region) the disease appeared when those countries started to import seed from North America (Gasperini *et al.*, 1982; Signoret, 1984). In 1978 EPPO listed Psg in the A2 quarantine list (Anonymous, 1978), but six years later, as it was reported in many areas to cause only small losses, Psg was deleted from the list. The increased importance of soybean in some European countries such as Italy, Spain and France in the 80's made the EU to keep the pathogen under quarantine legislation (EEC Directive, 1981); it was deleted in 1991, but was kept under quality regulations. Since the prevalence and severity of the disease varies considerably from year to year and from region to region, depending mostly on cultivar and environmental conditions (Eathington *et al.*, 1993) the question arose whether, under European cultural and climatic conditions, this disease might cause epidemics leading to significant yield losses. Most studies on the effect of bacterial blight have been done by experimentally inoculating soybean plants at various growth stages; since crop rotation is a normal practice in European countries only seedborne inoculum would likely be important (Graham, 1953; Daft and Leben, 1973). The aim of the present work was to investigate whether contaminated seed lots could represent a threat in European soybean-growing areas and to study a possible correlation between seed infection and disease severity, yield and the quality of the seed harvested.

MATERIALS AND METHODS

Bacterial strain. The strain of Psg GRISP 349 was used; it was isolated by J.F. Chauveau at the S.R.P.V., Angers (F) from a lot of infected soybean seed and proved to be highly virulent. Each year, prior to carrying out the experiments in the field, the strain was inoculated into soybean plants in a glasshouse and reisolated from blighted leaves to preserve its virulence.

Plants. Each participating lab used a set of soybean cultivars well known and adapted to the local conditions. In Italy these were 'Westfield', 'Gemma' and 'Crusader' in the first year, 'Westfield' and 'Gemma' the second year and 'Gemma' the third year; in France 'Apache', 'Labrador' and 'Crusader' in the first, 'Apache' and 'Labrador' in the second and 'Essoir' in the third year; in Spain the cultivars were 'Verdon' and 'Crusader' (1st year), 'Bolero' and 'Soynova' (2nd year), 'Futura' (3rd year). Each year the seed to be used was tested before the experiments and proved to be free from the pathogen; in glasshouse experiments all these cultivars proved to be susceptible to the strain used.

Seed inoculation. Soybean seeds were pregerminated on wet, sterile Whatman paper in Petri dishes at 25°C for 24 hours. Psg was grown on agar plates (YDC or King's B medium) for 48 hours, suspended in distilled water and adjusted spectrophotometrically to a final concentraton of approximately 2×10^8 cells ml^{-1}; the pregerminated seeds were pricked with a sterile needle three times and then dipped in the bacterial suspension and vacuum infiltrated for five minutes.

Field trials. Four European regions were chosen for field trials: the central Po Valley (Italy), the western Loire Valley (France), the Tago Valley and Andalusia (Spain). In the first year four contamination levels for each cultivar were considered: 0 (control), 0.5, 5 and 20%; the second and third year only three levels of seed contamination were made: 0 (control), 2 and 20%. For each cultivar, all treatments were distributed randomly in the plots. Each year 48 plots were sown at a density varying from 62 seeds m^{-2} in Italy, 50 seeds m^{-2} for France and 42 seeds m^{-2} for Spain. A plot was 8 m long x 8 rows large: plots were separated by three rows of maize (Italy) or three rows of sunflower (France and Spain) to minimize cross contamination from plot to plot by wind and/or rain splashes. *Bradyrhizobium japonicum* was used to inoculate the seed or the soil. Fields were visually inspected each week, starting from emergence; temperature, rainfall, humidity and leaf moisture were monitored throughout the growing period. Plant growth, phytosanitary state and the appearance of any bacterial disease symptom were assessed at each inspection.

Epiphytic Psg. From the plant growth stage V3 to R6 two to five samplings were taken. For each plot 25 mature and asymptomatic leaves were collected at random to detect the pathogen on leaves. In Italy, each leaf sample was washed by shaking in sterile 0.58% NaCl solution for two hours at room temperature; the washing fluid was then centrifuged (10 min at 10,000 ***g***), the pellet diluted in 0.5 ml of sterile water and 50 µl

streaked on nutrient sucrose agar (NSA) plates supplemented with Cephalexin (50 ppm) and Cycloheximide (40 ppm) in two replicates. Psg-like colonies were then transferred to King's B agar plates (King *et al.*, 1954) and identified. In France and Spain each leaf sample was homogenized in sterile water and, after filtration, the fluid was plated on King's B agar plates supplemented with the same antibiotics as in Italy, in two replicates. Psg-like colonies were purified and identified. In Italy identification was done by the indirect fluorescent antibody staining technique (Calzolari and Mazzucchi, 1990) using two sets of antisera; in France and Spain identification was performed according to LOPAT tests (Lelliott *et al.*, 1966), and also acid production from inositol, sorbitol, erythritol, mannitol (Ayers *et al.*, 1957) and utilization of DL-lactate, D(-) tartrate, L(+) tartrate.

Harvest and post-harvest analyses. The central part of each plot (4 central rows x 6 m long) was manually harvested and threshed; seed was cleaned and the productivity per ha was calculated at 14% seed humidity. The sanitary quality of the harvested soybeans was checked with IFAS (in Italy) and direct isolation (in France and Spain). For each plot, five replicates of 1000 seeds, five replicates of 100 seeds and five replicates of 10 seeds were analysed to estimate crop contamination rate using the most probable number (MPN) tables according to Swaroop (1951) and Taylor and Phelps (personal communication).

In Italy, seed contamination was also assessed just before harvesting by combined biological and enzymatic amplification (BIO-PCR) (Schaad *et al.*, 1995). Ten pods were taken at random from each field plot, keeping them in individual envelopes. Five seeds were aseptically removed and put in sterile Eppendorf tubes adding 1 ml of sterile PBS, pH 7.2. Tubes were shaken overnight on a rotary shaker at room temperature and 50 µl of the washing fluid was plated on NSA as above and grown for 3 days at 27°C. Plates were then washed with 2 ml of PBS and washes were used directly for PCR. Amplification was performed according to Ullrich *et al.* (1993) except for dimethylsulphoxide concentration (1% instead of 10%, v/v).

Statistical analyses. Linear regression was performed on data obtained from the epiphytic presence and spread of the pathogen. The data on productivity were submitted to principal component analysis and then analysed by analysis of variance (*F* test) (Steel and Torrie, 1980).

RESULTS

Italian experiments. The Psg strain GRISP 349 confirmed its high virulence in greenhouse pathogenicity tests on the soybean cultivars used.

The meteorological data recorded from sowing to harvest were quite normal for the period considered (Table 1), except the first two weeks of August, when the average temperatures were somewhat higher than normal: late spring and early summer (soybean stages V2 to R1) were rainy. Total rainfall for the growing period was 200 to 450 mm; only in the second year of experiments irrigation was applied, especially in view of the sandy soil, with low availability of plant nutrients. For long periods, especially in the first weeks after emergence, leaf moisture was often high enough to allow penetration of the pathogen from the leaf surface into the mesophyll.

The first symptoms of the disease appeared at random within the plots, more frequently affecting plants derived from seeds with the highest level of contamination. Generally symptoms were detected at the V3 stage and were typical for the disease. These lesions increased in number and size until stage R3-R4 and the distribution in the single plots of the affected plants was not completely even: more efficient disease progression was observed along the rows rather than crosswise. For the three cultivars used, disease progression was higher during the first stages up to stage R2, generally reached at the end of June.

Analyses performed to detect Psg in the epiphytic flora showed a general increasing level of leaf contamination as shown in Table 2 for the cvs 'Gemma' and 'Westfield'. Until stage R1-R2 the leaf contamination in the plots from seed with the highest contamination rate was significantly higher than in the control; later no significant differences were found among the three levels of initial seed contamination. Cv. 'Gemma' gave the highest leaf contamination rate, cv. 'Westfield' the lowest. The increase index of the epiphytic presence of Psg was significantly different at the higher seed contamination level for both cvs; the symptom index was also significantly higher (Table 2): the two indices appeared to be correlated. The percentage of diseased plants reached 5.4%±0.4 for the control plots, 7.2%±0.5 for the lowest seed contamination rate and 10.2%±0.8 for the highest seed contamination rate (cv. 'Gemma'). The last value was significantly higher than the control ($P = 0.05$).

Average yields for the individual tests are shown in Table 3: no significant differences were found among the three treatments, both considering the yearly results and the final means. In 1994 productivity was reduced by about the 28-30% due to the maize barriers sown

Table 1. Main climatic features from 1st June to 30th September in two European experimental areas. This period extended approximately from soybean emergence-V1 to harvest. Data were collected within the experimental fields.

ITALY

	Temperature (°C)			
Year	Average min.	Average max.	Total rainfall (mm)	Days with rain
1992	14.2	28.9	115.8	31
1993	14.0	28.8	189.6	32
1994	14.4	28.9	401.6	28

FRANCE

	Temperature (°C)			
Year	Average min.	Average max	Total rainfall (mm)	Days with rain
1992	13.6	27.7	194.3	27
1993	11.8	25.6	224.0	27
1994	10.1	31.5	187.0	16

too tightly around each plot, in order to prevent bacteria spreading from plot to plot. For the most susceptible cultivar 'Gemma', seed protein content and oil yield were also analysed but no significant differences were detected within the three tests examined.

Post-harvest phytosanitary analyses revealed a high degree of seed contamination; according to the most probable number (MPN) tables for the estimation of percentage seed infection the seed collected in the plots with an initial 20% seed contamination rate had an MPN=0.30; the seed collected in the plots with a 2% initial contamination rate had an MPN=0.25 and the seed collected from the control plots had an MPN=0.17. These values were significantly different.

PCR analyses of seed taken aseptically from pods before harvest revealed no contamination by Psg.

French experiments. On the soybean cultivars used in France over the three years the Psg strain proved highly pathogenic: after seed inoculation with Psg symptoms developed on an average of 93% of the plants in the glasshouse.

Over the three years the climate was almost normal for the region (Table 1), somewhat drier and warmer during the first stages of soybean development: total rainfall during the growing period was 120-250 mm. No particular event occurred (hail, storms, strong winds) during the stages of highest susceptibility to Psg. Two to six sprinkler irrigations were needed each year.

The germination rate in the field showed no difference between contaminated seed and the control.

Psg was first isolated on the epiphytic flora each year at stage V3, and only in infected plots, when no symptom was ever detected. Sampling at stage R2 revealed that in 90 to 100% of the contaminated plots Psg was present epiphytically, whereas it was present in 33% of the uncontaminated ones. First symptoms appeared on leaves at stage V4 and increased until stage V6-R1. The disease developed first in inoculated plots and, later, on some control plots as well; the percentage of plants showing symptoms at the last observation was 9-19% for the control, 23-26.4% for the low level inoculation treatment and 23.9-24% for the high level inoculation treatment, depending on year of experiment and cultivar. The index of epiphytic presence of Psg was significantly correlated with seed contamination rate only for cv. 'Essor': for the same cv. the symptom index was higher in the contaminated plots than in the controls (Table 2). As in the Italian field experiments, the epiphytic presence of Psg was correlated with symptom expression. In the first and second years correlation between symptoms, disease progression and seed contamination was highly significant. During the third year, which was the warmest, first symptoms appeared at the beginning of August (stage R3) after some days of showers, both on inoculated and uninoculated plants, at an average contamination rate of 0.7 to 1.2%. No symptom was ever detected on flowers or pods. In general disease progression was higher during rainy periods and faster in the early stages.

Table 2. Disease indices calculated plot per plot. Final symptom index was calculated after the last inspection at stage R5. The increase index of epiphytic Psg is the angular coefficient calculated for each treatment by means of linear regression. The increase index was calculated up to stage R1-R2.

ITALY

	Symptom index (diseased plots/total)			Increase index of epiphytic presence of Psg		
Cultivar	Control	2-5% cont.	20% cont.	Control	2-5% cont.	20% cont.
Gemma	0.375	0.562	1.000	2.5	4.0	7.0
Westfield	0.375	0.250	0.625	2.0	2.0	5.0

FRANCE

	Symptom index (diseased plots/total)			Increase index of epiphytic presence of Psg		
Cultivar	Control	2% cont.	20% cont.	Control	2% cont.	20% cont.
Essor	0.375	0.625	0.563	0.5	2.0	3.5
Apache	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Labrador	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s.: no significant difference compared to the control.

Yearly mean production for each cv. is shown in Table 3. Variance analysis performed separately for each cv. showed no significant difference in the production between infected and uninfected plots. Depending on the cultivar and year of experiment Psg was detected in 25 to 100% of the harvested seed from contaminated plots and in 0 to 25% of the control plots. The presence of the pathogen on the harvested seed was significantly correlated with the rate of seed infection before sowing. Susceptibility measured as percentage of diseased plants did not show any difference among the different cultivars used: from the harvested seed Psg could be isolated more often from cv. 'Labrador' and less often on cv. 'Apache'.

Concerning the quality of the harvested seed the calculated MPN for the percentage of seed contamination was between 0.0 and 0.1, thus not significantly different among the three treatments.

Spanish experiments. Reisolation, purification and pathogenicity tests with Psg gave the same results for the Spanish soybean cultivars as for the tests done in Italy and France. In Spain soybean was sown as second crop each year. The weather during the experiments was quite different from that in Italy and France since the Spanish areas had very dry conditions and higher temperature, often 35-40°C and more. Such high temperatures were never reached in the other two countries. Sprinkler irrigation was used at least 5-6 times each growing season. Nevertheless no symptom of the disease was seen and the pathogen was never reisolated from the epiphytic flora.

The mean yields in the individual tests are shown in Table 3; seed contamination rate was not correlated with productivity in the three cultivars used. The common cv. 'Crusader' yielded a significantly lower amount of seed than when grown in France or Italy: in Spain production was reduced to about one third. Post-harvest analyses of the seed never revealed any seed contamination.

DISCUSSION

Soybean has been grown in Italy, France and, to a lesser extent, in Spain since the early eighties. Bacterial blight has been regularly observed since the crop became extensively grown in northern Italy, although serious yield losses have never been reported.

The experiments highlighted two important features in the epidemiology of bacterial blight of soybean: importance of the seed-borne inoculum and dependence of disease intensity on the climatic conditions and the cultivar used. Italian and French results showed that the epiphytic phase of the pathogen and the appearance of the disease in the field, at the beginning of the monitoring, were related to the percentage of seed contamination. It is common to find the pathogen in seed lots imported from countries where Psg is present and widespread (Calzolari, 1992). Environmental conditions affect increase and activity of epiphytic populations of

Table 3. Soybean productivity in three EU-Countries in the years 1992-94. The seed was experimentally inoculated at different rates with *P. syringae* pv. *glycinea* just before sowing. Cultivation conditions were normal for the area of production considered. After threshing, productivity was calculated in q ha^{-1} at 14% of relative seed humidity.

ITALY

	1992			1993			1994*		
Cultivar	Control	2-5%	20%	Control	2-5%	20%	Control	2-5%	20%
Gemma	36.85	40.65	35.12	30.21	27.50	30.21	24.78	24.78	25.03
Westfield	37.45	33.68	38.25	27.50	28.54	28.13			
Crusader	36.90	34.22	34.68						

FRANCE

	1992			1993			1994		
Cultivar	Control	2-5%	20%	Control	2-5%	20%	Control	2-5%	20%
Apache	38.39	38.04	36.83	35.61	36.20	36.59			
Labrador	35.73	34.81	38.33	33.95	33.31	33.79			
Crusader	35.18	35.77	34.81						
Essor							25.70	26.60	26.80

SPAIN

	1992			1993			1994		
Cultivar	Control	2-5%	20%	Control	2-5%	20%	Control	2-5%	20%
Crusader	23.32	22.90	22.07						
Verdon	24.75	24.01	29.81						
Soynova				22.45	25.11	24.98			
Bolero				26.36	23.80	23.44	28.27	28.13	27.90

* Productivity was reduced by the 28-30%, compared to the mean productivity of cv. 'Gemma' in that area, by competition from maize barriers surrounding each individual plot.

bacteria (Sigee, 1993) and weather conditions conducive to the disease in our Italian and French experiments allowed pathogen populations to increase and develop symptoms. Spring rains and summer showers easily spread the pathogen throughout the field, in some cases also to some control plots. In a few cases, no significant differences were found between contaminated plots and controls indicating the possibility that the pathogen could have been spread beyond the maize (or sunflower) barriers during windy showers.

Between emergence and flowering the general pattern of spread is the same as described by Van der Plank (1963): at stages V3-V4 the disease is confined to focal outbreaks, mostly located in the contaminated plots. In the following stages generalised disease with many small foci develops. Heavy spring rainfall and prolonged period of leaf wetness characterize Italian and French areas and favoured the multiplication and spread of Psg; high summer temperature and scanty rainfall caused a drastic drop in the epiphytic population of the pathogen, slowing down the appearance of symptoms by hindering survival of the pathogen in the phylloplane as well as its penetration into the host, and confirming the importance of weather conditions for epidemics. In Spain, high temperature and low humidity prevented colonization of the plant surface and appearance of symptoms, even in the case of heavy seed contamination. Frequent sprinkler irrigation seemed to be not sufficient to allow the survival and the penetration of Psg into the host plant, probably because leaf moisture did not last long enough to allow bacterial penetration into the mesophyll through the stomata.

In our experiments, unlike other pseudomonads causing leaf spot, Psg did not become systemic. The disease was strongly conditioned by meteorological factors and, in particular, did not spread at high temperatures and in the concomitant absence of rain. Under these conditions the epiphytic bacterial population, which could remain below the detection limit, was markedly reduced.

Most of the epidemiological studies and the experiments on yield losses described in the literature deals with the contamination of plants or plantlets after emergence one or more times resulting in heavy defoliation and yield reduction (Nicholson *et al.*, 1973; Williams and Nywall, 1980). Experimental contamination of open flowers may lead to seed infection (Leben, 1976). Our experiments tended to simulate more natural conditions; despite the possibility for Psg to colonize the plant surface in the early stages, when the temperatures are not too high and the mobility of the pathogen is most active (Hatterman and Ries, 1989) the disease did not cause defoliation, but only leaf spots. Higher temperature and subsequent decrease of epiphytic population at flowering time may have prevented efficient flower colonization so that no bacteria were found inside the pods. From contaminated plots we regularly found contaminated seed, and the contamination probably occurred during threshing, through dust and plant debris.

In Italy a positive correlation between level of seed infection, presence of epiphytic Psg and phytosanitary quality of the harvested seed was found, both for the more susceptible cv. 'Gemma' and for cv. 'Westfield'. Even in the absence of a significant loss of product, the possibility is confirmed that seed quality will deteriorate when levels of natural inoculum increase. This could then lead to an epidemic when there is a combination of highly susceptible cultivar and favourable climatic-environmental conditions, for example during a rainy summer, with significant losses, as already reported in some areas of the United States (Eathington *et al.*, 1993) or in case of multiple infection as in the synergistic interaction between *Diaporthe phaseolorum* var. *caulivora* and Psg (Pacumbaba, 1992).

The Italian results showed that the post-harvest contamination is external, and suggest the possibility to reduce seed contamination by disinfecting the seed externally by means of copper compounds or chlorine, in order to reduce the risk of possible epidemics.

The Spanish results highlighted the point that even in case of heavily contaminated seed, weather and cultural conditions may inhibit plant colonization by the pathogen. The post-harvest analyses confirmed the good phytosanitary quality of the seed produced in the Spanish regions, suggesting the choice of such areas to produce Psg-free seed.

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