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# Effect of *Pseudomonas syringae* subsp. *syringae* on yield and biomass distribution in wheat

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## Abstract

The effect of *Pseudomonas syringae* subsp. *syringae* on seed yield, aerial biomass production and partitioning in wheat (*Triticum aestivum* L.) is unknown. A field experiment was carried out in two locations of the Mexican Highlands (Montecillo and Chapingo) to evaluate the response of two wheat cultivars ('Seri M82' and 'Rebeca F2000') to four inoculum rates ($10^{6, 8, 10}$ cfu $mL^{-1}$, plus a control without inoculum). Disease incidence and severity, seed yield, seed number and seed size were measured. At flowering and seed physiological maturity, aerial biomass production and distribution of main stem, secondary stems and total plant were recorded. Source-sink relationships during the grain filling period were estimated. Higher values of disease incidence and severity were observed at Chapingo; the same traits were also greater in 'Seri' than in 'Rebeca' at both sites ($p < 0.05$). Seed yield, seed number and seed size of 'Rebeca' were higher ($p < 0.05$) than that of 'Seri'. The pathogen reduced ($p < 0.05$) plant height, seed yield, seed yield components, and biomass production of most organs of main and secondary stems. The magnitude of the reductions was similar in both cultivars at both sites. The effect of the bacteria at each location was higher ($p < 0.05$) at greater doses affecting seed number more than seed weight. Stems prevailed as sink organs, while laminae, sheaths, spikes, and other vegetative parts predominated as source organs. Plant disease records should complement crop physiological variables to evaluate and to explain bacterial disease effects.

**Additional key words:** plant diseases; plant pathogenic bacteria; source-sink relationships; *Triticum aestivum*.

## Resumen

**Efecto de *Pseudomonas syringae* subsp. *syringae* en el rendimiento y distribución de biomasa en trigo**

El efecto de *Pseudomonas syringae* subsp. *syringae* en el rendimiento de semilla y en la producción y distribución de biomasa aérea en trigo (*Triticum aestivum* L.) es desconocido. Se sembró un experimento en dos localidades de los Valles Centrales de México (Montecillo y Chapingo) para evaluar la respuesta de dos cultivares de trigo ('Seri M82' y 'Rebeca F2000') a cuatro dosis de inóculo ($10^{6, 8, 10}$ cfu $mL^{-1}$, más un testigo sin inóculo). Se registraron datos de incidencia y severidad, rendimiento de semilla y número y tamaño de semilla. En la floración y madurez fisiológica de semilla, se registró la producción y distribución de biomasa aérea de tallos, vainas, limbos y espigas de tallos principales, secundarios y total por planta. Se estimaron las relaciones fuente-demanda durante el periodo de llenado de grano. La incidencia y severidad de la enfermedad fueron mayores en Chapingo, y en 'Seri' respecto a 'Rebeca' en ambas localidades ($p < 0,05$). El rendimiento de semilla, así como el número y tamaño, fue mayor ($p < 0,05$) en 'Rebeca' que en 'Seri'. El patógeno redujo ($p < 0,05$) la altura de la planta, el rendimiento y sus componentes, y la producción de biomasa de la mayoría de los órganos de los tallos principales y secundarios; sin embargo, la magnitud de las reducciones fue similar en ambos cultivares. El efecto de la bacteria en cada localidad fue mayor ($p < 0,05$) a dosis mayores al afectar al número de semillas más que al peso de las mismas. Los tallos prevalecieron como órganos de demanda, mientras que limbos, vainas, espigas, y otras partes vegetativas predominaron como órganos fuente. Los reportes de

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enfermedades de las plantas podrían complementarse con variables fisiológicas del cultivo para evaluar y explicar los efectos de las enfermedades bacterianas.

**Palabras clave adicionales:** bacteria fitopatógena; enfermedades de plantas; relaciones fuente demanda; *Triticum aestivum*.

## Introduction

Plant pathogenic bacteria may reduce seed yield depending upon environmental factors prevailing at the time of the infection, the growth stage of the host crop, and cultivar tolerance, among others. Evaluations of bacteria damage in wheat (*Triticum aestivum*, *T. durum*) seed yield, with or without controlled inoculation and conducted under field conditions are scarce (Duveiller and Maraite, 1993). In these studies, when seed yield is measured, yield components (seed number and seed size) affected are not mentioned.

The incidence of *Pseudomonas syringae* pv. *syringae* (*Pss*) on wheat is generally sporadic, although yield losses can be devastating when environmental conditions are favorable for epidemic development (Duveiller *et al*., 1993) in susceptible hosts. In USA, Schaad and Forster (1985), Forster and Schaad (1988) and Tillman *et al*. (1996) reported around 40% of yield losses under field conditions due to *Xanthomonas translucens*. In Europe and Asia yield losses reached up to 50% (von Kietzell *et al*., 1994). In warm and humid environments of the State of México, *X. translucens* reduced grain yield of wheat cv. 'Alondra' by 10 to 20% (Duveiller and Maraite, 1993).

In the Central Mexican Highlands wheat is sown under rainfed conditions (Hernández *et al*., 1998). In this region, *Pss* has not been detected causing severe damage, but its incidence and severity have increased in recent years. Valencia-Botín *et al*. (2007) showed that severity data on wheat seedlings inoculated with *Pss* were more reliable when dry weight was measured than data symptoms based on leaf area damage. These authors proposed to evaluate the damage caused by plant pathogenic bacteria directly on yield, to complement the routine severity assessments.

The biomass accumulated in plant organs results from physiological processes, as photosynthesis and distribution of assimilates. In cereals, yield is usually more associated with seed number than with seed size. Thus, it is expected that the presence of an adverse biotic factor (*e.g.* pathogenic bacteria) during flower differentiation will have more impact on seed number than on seed size (González and Grimaldo, 1991). In turn, most of the biomass fraction accumulated in the seed is generated during the post-anthesis period. However, under environmental stress (*e.g.* water deficit) seed biomass may come from pre-anthesis photosynthesis, *e.g.* the mobilization of reserves accumulated in stems, leaf sheaths or vegetative structures of the spike (Lopez-Castañeda and Richards, 1994; Valadez-Gutiérrez *et al*., 2006). The difference in dry weight accumulated in organs at physiological maturity with respect to that accumulated at flowering could be useful to evaluate sink-source relationships. In bread wheat, yield reductions were found even under slight damages (*e.g.* defoliation, or mottling of laminae or stems) caused by pathogens (Zamsky and Schaffer, 1996; Espitia and Villaseñor, 2000; Solís-Moya *et al*., 2007) but the effect of plant pathogenic bacteria in sink-source relationships of wheat has not been studied.

In order to evaluate the effect of four rates of inoculum of *Pss* inoculated at the seedling stage on seed yield and production and distribution of post-anthesis biomass, two wheat cultivars (one presumably bacteria resistant and the other susceptible) were sown in two locations of the State of México, México. It was expected that reductions in biomass production, seed yield, and seed yield components are better estimates of the bacterial damage than pathogen incidence and severity records alone. Also, it was hypothesized that sink-source relationships will be modified by the pathogen, and that seed yield and biomass production will decrease more in the susceptible cultivar than in the resistant.

## Material and methods

The study was conducted in 2004 at Montecillo and Chapingo, both located in the State of México. Montecillo is located at 19°29' N and 98°53' W, 2250 m altitude, climate C(i)B/2(a'), annual average temperature of 14.6°C and 558 mm average annual rainfall. Chapingo is situated in the same coordinates as Montecillo, but its climate is more humid and slightly

Abbreviations used: I (incidence); MS (main stem); *Pss* (*Pseudomonas syringae* pv. *syringae*); S (severity); SN (seed number); SS (secondary stem); TP (total plant; MS+SS); 100SW (100-seeds weight).

cooler: Cb(wo)(w)(i')g, average annual temperature of 15.2°C and 636 mm rainfall (García, 1988).

Eight treatments were evaluated at each location. Treatments were the factorial combination of two wheat cultivars, 'Seri M82' (thereafter named 'Seri', susceptible to *Pss*) and 'Rebeca F2000' (thereafter named 'Rebeca', presumably pathogen tolerant) (Villaseñor *et al*., 2004) and four inoculum doses of *Pss* ($10^6$, $10^8$, $10^{10}$ cfu $mL^{-1}$ and a negative control, without *Pss*). Treatments were allocated in a complete random block design with four replications. The experimental plot was four rows, 3 m long, 0.30 m apart, and 0.10 m between plants.

Sowing dates were June 23 at Montecillo and July 5 at Chapingo. At Chapingo, weeds were controlled with Esteron 47-M (2, 4-D 400 g a.i. $ha^{-1}$), and at Montecillo with manual weeding. Folicur 250 EC (tebuconazole 125 g a.i. $ha^{-1}$) was applied to prevent rust incidence. In both locations, plots were fertilized with 80-40-00 NPK $ha^{-1}$: 40-40-00 at sowing and the remaining 27 d later. A single watering was applied at seedling emergence because rainfall was satisfactory for plant growth at each location.

The inoculum was applied to seedlings with a manual sprayer (Swissmex-Rapid ® Mod 310) during growth stage 15 (GS15) (five laminae on the main stem) (Zadoks *et al*., 1974).

Incidence (I) and severity (S) data were recorded 22 d after inoculation when plants were at stage 55 (half of the panicle had emerged). The incidence and severity was recorded in the laminae of the main stems of all plants in the plot. Incidence was the percentage of main stems showing any symptoms of stem blight. Severity was the average of diseased tissue of the flag leaf (from 1% to 75%) according to the scale proposed by Duveiller (1994).

At flowering, three fully competitive plants per plot were marked and the length of their main and secondary stems (from ground level to spike apex) was measured. In addition, the main and secondary stems of three plants were cut and total dry aerial biomass and its distribution in stems, sheaths, laminae and spikes per plant were weighed. These organs were dried in an oven at 70°C for 72 h. At seed physiological maturity (black layer present in seeds at the bottom of the main panicle in 50% of the panicles of secondary stems), the production and allocation of aboveground dry biomass in three plants per plot were also measured as already described.

Seed yield (separating both, the main stem spike and the secondary stems spikes) was recorded. The respective spikes were cut, dried and weighed before and after threshing, so once seed weight was separated, the difference between both measurements was biomass allocated in vegetative structures of the spike (sum of rachis, lemma, palea, glumes and awns). Afterwards, seed yield components were registered: 100-seeds weight (100SW, g, of a randomly selected sample), and seed number (SN) from the main stem spike and for all secondary spikes, were measured. Plant seed yield was the sum of that from main stem and secondary stems.

Dry weight differences between samplings at physiological maturity and flowering corresponded to gains or losses of biomass during grain growth for each organ. These differences were used to estimate sink-source relationships during the grain filling period.

In each location a complete randomized blocks experimental design was setup with four replicates in a 4×2 factorial arrangement of treatments. Individual analysis of variance (ANOVA, PROC GLM) was made for each location (SAS Institute, 1999). Variables expressed in percentage (*Yi*) were transformed through the arcsine $[(Yi)^{1/2}]$. Mean comparisons were performed with the Tukey test ($p < 0.05$).

## Results and discussion

Individual ANOVA analysis (Table 1) indicated that except for 100-seeds weight in main stems at Chapingo, significant differences ($p < 0.05$) were found for all variables among cultivars (C) and doses (D). The C × D interaction was significant in 17 out of 28 cases (61 %), and variation coefficients ranged from 3 to 13%, except for bacterial incidence and severity at Montecillo where it was 37%.

### Bacterial incidence and severity

As expected, averaged over inoculum doses, incidence and severity of *Pseudomonas syringae* (*Pss)* were higher ($p < 0.05$) in 'Seri' than in 'Rebeca' at each location, confirming the assumed resistance of 'Rebeca' (Villaseñor and Espitia, 2000). Overall, incidence and severity values were higher ($p < 0.05$) as inoculum doses increased in both locations (Table 2).

In the individual ANOVA, C × D interaction was significant ($p < 0.05$) for bacterial severity at each location and for disease incidence only at Chapingo (Table 1). Thus, both traits did not have a similar response; for instance, at Chapingo at the highest inoculum dose 'Seri' had higher incidence ($p < 0.05$) than 'Rebeca', but at the low dose both cultivars showed

**Table 1.** Mean squares and significance[1] of sources of variation for plant length, seed number, seed yield, 100-seeds weight, bacterial incidence and severity of two wheat cultivars in main stems, secondary stems and plant total

| Variable | Cultivar (C) | Dose (D) | C × D | CV (%)[2] |
|---|---|---|---|---|
| *Main stems at Montecillo* | | | | |
| Plant length (cm) | 1095.30** | 53.94** | 15.11 ns | 5 |
| Seed number | 102.45** | 244.40** | 10.35** | 3 |
| Seed yield (g) | 0.51** | 3.40** | 0.03ns | 7 |
| 100-seeds weight (g) | 3.95** | 0.29** | 0.14* | 5 |
| Bacterial incidence (%) | 268.47** | 555.98** | 51.76 ns | 37 |
| Bacterial severity (%) | 401.37** | 625.24** | 67.22* | 37 |
| *Main stems at Chapingo* | | | | |
| Plant length (cm) | 1239.15** | 115.21** | 66.69** | 3 |
| Seed number | 33.21* | 331.84** | 19.22* | 7 |
| Seed yield (g) | 34.22** | 32.05** | 4.91** | 5 |
| 100-seeds weight (g) | 0.22ns | 0.55** | 0.10 ns | 9 |
| Bacterial incidence (%) | 243.91** | 1112.12** | 43.22** | 11 |
| Bacterial severity (%) | 108.18** | 1735.91** | 28.32** | 7 |
| *Secondary stems at Montecillo* | | | | |
| Plant length (cm) | 1331.41** | 34.73** | 10.61 ns | 3 |
| Seed number | 17662.60* | 8183.01* | 507.24 ns | 6 |
| Seed yield (g) | 9.69** | 47.21** | 2.88** | 3 |
| 100-seeds weight (g) | 0.59** | 0.88** | 0.29** | 8 |
| *Secondary stems at Chapingo* | | | | |
| Plant length (cm) | 1020.96** | 26.05* | 13.91 ns | 5 |
| Seed number | 323893.77** | 114849.34** | 19031.33** | 11 |
| Seed yield | 127.16** | 68.39** | 9.66** | 3 |
| 100-seeds weight (g) | 2.18** | 0.16** | 0.01 ns | 5 |
| *Total per plant at Montecillo* | | | | |
| Plant length (cm) | 1344.86** | 63.68 ns | 11.97 ns | 7 |
| Seed number | 24144.48** | 13015.89** | 473.17 ns | 13 |
| Seed yield (g) | 14.66** | 76.01** | 2.86** | 2 |
| 100-seeds weight (g) | 1.79** | 0.97** | 0.13** | 4 |
| *Total per plant at Chapingo* | | | | |
| Plant length (cm) | 1115.69** | 57.90** | 28.28** | 3 |
| Seed number | 324469.50** | 124100.73** | 17540.86** | 9 |
| Seed yield(g) | 294.64** | 194.27** | 27.46** | 4 |
| 100-seeds weight (g) | 0.95** | 0.32** | 0.02 ns | 3 |

[1] *, **: significant at 5 and 1 %, respectively. [2]CV = coefficient of variation.

similar incidences (Table 2). Regarding disease severity, again at Chapingo, 'Rebeca' had lower values than 'Seri' ($p < 0.05$) in the three inoculum doses. Furthermore, at Montecillo, 'Seri' showed the highest incidence and severity, while 'Rebeca' had high incidence when a high dose was applied but its severity values were similar in all inoculated plants (Table 2). This suggests that: (a) when bacterial diseases are assessed on wheat, a direct association between incidence and severity does not always prevail, particularly when more than one location is involved in the evaluation, and (b) bacteria found more barriers to cause damage in 'Rebeca' than in 'Seri'.

## Main stems

Main stem (MS) length of 'Seri' was lower ($p < 0.05$) than 'Rebeca' at each location, but *Pss* caused between 3 and 7% reduction in 'Seri', while decreases in 'Rebeca' were from 1 to 17%. This was particularly evident at Chapingo and at the highest dose (Table 3). Thus,

**Table 2.** Incidence (%) and severity (%) of *Pseudomonas syringae* on two wheat cultivars, inoculum doses (cfu mL$^{-1}$) and their interaction, in two locations of the State of México

| Cultivar | Montecillo | | | | | Chapingo | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $10^{10}$ | $10^{8}$ | $10^{6}$ | Control | Mean | $10^{10}$ | $10^{8}$ | $10^{6}$ | Control | Mean |
| *Incidence* | | | | | | | | | | |
| Seri | 21.4a* | 18.9a | 17.7abc | 0.0d | 14.5A | 29.8a | 29.0a | 21.2b | 0.0c | 20.0A |
| Rebeca | 18.3ab | 8.2cd | 8.3bcd | 0.0d | 8.7B | 21.7b | 18.6b | 17.6b | 0.0c | 14.5B |
| Mean | 19.9A | 13.6B | 13.0B | 0.0C | 11.6 | 25.8A | 23.8A | 19.4B | 0.0C | 17.2 |
| *Severity* | | | | | | | | | | |
| Seri | 26.4a | 22.0ab | 17.2ab | 0.0c | 16.5A | 38.3a | 27.6b | 25.4c | 0.0e | 22.8A |
| Rebeca | 12.9b | 12.2b | 12.5b | 0.0c | 9.4B | 29.9b | 26.2c | 20.5d | 0.0e | 19.2B |
| Mean | 19.7A | 17.1A | 15.1A | 0.0B | 13.0 | 34.1A | 26.9B | 23.0C | 0.0D | 21.0 |

*Means with different lower-case letter for C×D interaction or with different capital letter between cultivar or doses are different (Tukey, $p < 0.05$).

**Table 3.** Plant length, seed yield and yield components in main stems of two wheat cultivars, inoculum doses and their interaction, in two locations in the State of México

| Cultivar | Montecillo | | | | | Chapingo | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $10^{10}$ | $10^{8}$ | $10^{6}$ | Control | Mean | $10^{10}$ | $10^{8}$ | $10^{6}$ | Control | Mean |
| *Plant length (cm)* | | | | | | | | | | |
| Seri | 57.5d[1] | 58.3d | 58.8d | 62.0cd | 59.2B | 57.5d | 57.5d | 57.5d | 57.5d | 57.5d |
| | (7)[2] | (6) | (5) | (0) | | (4) | (4) | (4) | (4) | (4) |
| Rebeca | 69.2bc | 75.8ab | 76.3ab | 77.1a | 74.6A | 63.8bc | 63.8bc | 63.8bc | 63.8bc | 63.8bc |
| | (10) | (2) | (1) | (0) | | (17) | (17) | (17) | (17) | (17) |
| Mean | 63.3B | 67.1B | 67.5AB | 69.6A | | 60.7B | 62.0B | 67.7A | 67.9A | |
| | (9) | (4) | (3) | (0) | | (11) | (9) | (1) | (0) | |
| *Seed yield (g)* | | | | | | | | | | |
| Seri | 1.43d | 2.20c | 2.24bc | 3.13a | 2.25B | 1.02f | 1.93e | 2.77d | 3.90c | 2.41B |
| | (54) | (30) | (28) | (0) | | (74) | (51) | (29) | (0) | |
| Rebeca | 1.79d | 2.35bc | 2.60b | 3.27a | 2.50A | 1.57e | 3.02d | 5.41b | 7.91a | 4.47A |
| | (45) | (28) | (21) | (0) | | (80) | (62) | (32) | (0) | |
| Mean | 1.61C | 2.28B | 2.42B | 3.20a | | 1.29D | 2.47C | 4.10b | 5.90A | |
| | (50) | (29) | (24) | (0) | | (78) | (58) | (31) | (0) | |
| *Seed number* | | | | | | | | | | |
| Seri | 32.6f | 41.3d | 42.7cd | 45.4bc | 40.5B | 26.6d | 25.8d | 37.8a | 40.6a | 32.7B |
| | (29) | (10) | (6) | (0) | | (35) | (37) | (7) | (0) | |
| Rebeca | 37.2e | 41.6d | 48.2ab | 49.3a | 44.1A | 28.6cd | 32.1bc | 37.0ab | 41.1a | 34.7A |
| | (25) | (16) | (2) | (0) | | (30) | (22) | (10) | (0) | |
| Mean | 34.8D | 41.4C | 45.5B | 47.4A | | 27.6C | 29.0C | 37.4B | 40.8A | |
| | (26) | (13) | (4) | (0) | | (32) | (29) | (9) | (0) | |
| *100-seeds weight (g)* | | | | | | | | | | |
| Seri | 2.39e | 2.76de | 2.87bc | 3.16bc | 2.79B | 2.60bc | 2.57c | 2.72abc | 3.28ab | 2.79A |
| | (24) | (13) | (9) | (0) | | (21) | (22) | (17) | (0) | |
| Rebeca | 3.45ab | 3.43ab | 3.59ab | 3.69a | 3.49a | 2.81abc | 2.71abc | 3.15ab | 3.16ab | 2.96A |
| | (7) | (7) | (3) | (0) | | (11) | (14) | (0.3) | (0) | |
| Mean | 2.91C | 3.09BC | 3.19AB | 3.38A | | 2.70B | 2.64B | 2.93AB | 3.22A | |
| | (14) | (9) | (6) | (0) | | (16) | (18) | (9) | (0) | |

[1] Means with different lower-case letter for C × D interaction or with different capital letter between cultivars or doses are statistically different (Tukey, $p < 0.05$). [2] Number within parenthesis indicates percentage of reduction compared to control.

reductions in plant length were not associated with disease incidence and severity rating in this study. Concerning seed yield at Montecillo, where disease incidence and severity were lower, MS of the negative control plants of both cultivars showed the same yield (3.13 *vs*. 3.27 g). Although seed yield decreased as the inoculum dose increased, reduction percentages were similar in both cultivars, varying from 24 to 50% at Montecillo. This means that the assumed pathogen resistance of 'Rebeca' (Villaseñor and Espitia, 2000) did not necessarily result in a lower seed yield reduction at Montecillo. In contrast, at Chapingo headquarters of the wheat breeding program where 'Rebeca' was developed (Villaseñor *et al*., 2004), this cultivar expressed its whole yield potential because their main stems produced two-fold those of 'Seri' (7.91 *vs*. 3.90 g, on average). However, since yield of both cultivars decreased in a similar extent (between 31 and 78%, on average), high disease incidence and severity values shown by 'Seri' do not mean that in this cultivar yield decreases more than 'Rebeca' (regarded as bacterial resistant). This indicates that disease resistance, usually evaluated in terms of a low frequency of plants with symptoms and less foliar damage, is not necessarily associated with lower seed yield reductions of diseased stems (Valencia-Botín *et al*., 2007). The need to complement yield data with disease incidence and severity grades is clear in this study.

As for seed yield components, main stems of 'Rebeca' control plants had higher SN and 100SW that 'Seri' at Montecillo, but without differences ($p < 0.05$) at Chapingo (Table 3). Concerning dose effects, as the dose increased SN and 100SW decreased, but reductions of 100SW were lower than that of SN ($R^2 = 0.71$ in Montecillo and $R^2 = 0.77$ in Chapingo). This response could be attributed to *Pss* inoculation carried out during pre-anthesis, where floral structures are differentiating and growing (Slafer and Calderini, 2003). However, due to C × D interaction there was a differential dose effect on both yield components depending upon the cultivar.

## Secondary stems

Although *Pss* was inoculated when only the main stems were visible (GS15), bacteria symptoms were also evident in the secondary stems (SS) (Table 4) probably due to physical contact between the inoculated and non-inoculated leaves. Similar to main stems results (Table 3) length average of SS (Table 4) was higher ($p < 0.05$) in 'Rebeca' than 'Seri' at each location; however, inoculum effect was too small since maximum reduction (10%) occurred in 'Rebeca' at the highest dose in Chapingo.

Regarding seed yield of secondary stems in control plants, 'Rebeca' produced more ($p < 0.05$) than 'Seri' (11.21 *vs*. 9.14 g at Montecillo and almost twice at Chapingo, 13.77 *vs*. 6.91 g). It was again observed that although seed yield reductions were greater as inoculum dose increased, reduction percentages were similar in both cultivars, especially at the highest dose at Chapingo. The largest seed yield of secondary stems of 'Rebeca' coincided with maximum average seed number in both locations (286.4 *vs*. 239.4 at Montecillo; and 436.6 *vs*. 235.3 at Chapingo) and with the highest 100SW of this cultivar (2.96 *vs*. 2.69 g at Montecillo and 3.08 *vs*. 2.56 g at Chapingo). Similarly, reductions in SN due to inoculum doses were relatively lower than 100SW at Montecillo.

## Total per plant

The parallelism of plant height, seed yield and yield components from the main and secondary stems caused that whole plant data (data not shown) ratify: (a) greater plant length of 'Rebeca' at both locations and any inoculum dose, (b) small inoculum effects on stem length, (c) higher yield potential of 'Rebeca', mainly at Chapingo, although at this location, the highest inoculum dose caused seed yield reductions close to 72% of the control in this cultivar, (d) higher SN and 100SW in 'Rebeca' than 'Seri' in each location, and a trend to decrease seed yield components as inoculum dose increases, (e) the need to corroborate if disease incidence and severity values are correlated with reductions in seed yield and yield components.

## Effect of *Pss* on dry biomass production and distribution during the grain filling period

There were significant effects ($p < 0.05$) of the sources of variation in the biomass of most plant organs (stem weight does not contain seed production) from main and secondary stems and total yield per plant at both locations and samplings (Table 5).

**Table 4.** Plant length, yield and yield components in secondary stems of two wheat cultivars, inoculum doses and their interaction in two locations in the State of México

| Cultivar | Montecillo | | | | | Chapingo | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $10^{10}$ | $10^{8}$ | $10^{6}$ | Control | Mean | $10^{10}$ | $10^{8}$ | $10^{6}$ | Control | Mean |
| *Plant length (cm)* | | | | | | | | | | |
| Seri | 58.52c* | 28.7c | 59.3c | 61.6c | 59.6B | 55.3c | 56.1c | 56.0c | 56.7c | 56.0B |
| | (5)** | (5) | (4) | (0) | | (2) | (1) | (1) | (0) | |
| Rebeca | 68.1b | 72.8a | 74.0a | 74.9a | 72.4A | 64.6b | 66.0ab | 66.9ab | 71.8a | 67.3A |
| | (9) | (3) | (1) | (0) | | (10) | (8) | (7) | (0) | |
| Mean | 63.3B | 65.8AB | 66.7A | 68.3A | | 60.0B | 61.1AB | 61.5AB | 64.2A | |
| | (7) | (4) | (2) | (0) | | (7) | (5) | (4) | (0) | |
| *Seed yield (g)* | | | | | | | | | | |
| Seri | 3.18f | 6.60d | 7.58c | 9.14b | 6.62B | 2.47f | 3.85e | 4.83d | 6.91c | 4.52B |
| | (65) | (28) | (17) | (0) | | (64) | (44) | (30) | (0) | |
| Rebeca | 5.37e | 6.91d | 7.42c | 11.21a | 7.72A | 4.53d | 6.60c | 9.11b | 13.77a | 8.50A |
| | (52) | (38) | (34) | (0) | | (67) | (52) | (34) | (0) | |
| Mean | 4.27D | 6.75C | 7.50B | 10.1A (0) | | 3.50D | 5.22C | 6.97B | 10.34A | |
| | (58) | (34) | (26) | | | (66) | (50) | (33) | (0) | |
| *Seed number* | | | | | | | | | | |
| Seri | 210.3b | 227.6ab | 242.1ab | 273.6ab | 239.4B | 133.2e | 239.2cd | 253.9cd | 315.1bc | 235.3B |
| | (23) | (17) | (12) | (0) | | (58) | (24) | (20) | (0) | |
| Rebeca | 248.0ab | 260.6ab | 305.4a | 332.0a | 286.4A | 223.6d | 387.6b | 543.2a | 591.9a | 436.6A |
| | (25) | (22) | (8) | (0) | | (62) | (35) | (8) | (0) | |
| Mean | 231.1B | 244.1AB | 273.6AB | 302.8A | | 178.4D | 313.4C | 398.6B | 453.5A | |
| | (24) | (20) | (10) | (0) | | (61) | (31) | (12) | (0) | |
| *100-seeds weight (g)* | | | | | | | | | | |
| Seri | 2.19c | 2.37bc | 2.61bc | 3.59a | 2.69B | 2.43d | 2.47d | 2.66cd | 2.67cd | 2.56B |
| | (39) | (34) | (27) | (0) | | (9) | (8) | (1) | (0) | |
| Rebeca | 2.54bc | 2.74b | 3.25a | 3.34A | 2.96A | 2.89bc | 2.99ab | 3.17ab | 3.26a | 3.08A |
| | (24) | (18) | (27) | (0) | | (11) | (8) | (3) | (0) | |
| Mean | 2.37C | 2.55C | 2.92B | 3.46A | | 2.66C | 2.73BC | 2.91AB | 2.96A | |
| | (32) | (26) | (16) | (0) | | (10) | (8) | (2) | (0) | |

* Means with different lower-case letter for C×D interaction, or with different capital letter between cultivars or doses are statistically different (Tukey, $p < 0.05$). ** Number within parenthesis indicates percentage of reduction compared to control.

Biomass production in plant organs, samplings and locations, as well as gains or losses (increases or decreases in biomass) during the seed filling period is shown in Figure 1. Gains indicate that a particular organ served as a sink during post-anthesis; losses indicate that the organ functioned hypothetically as a source during that period since main source tissues, like mature leaves, produce assimilates sent to sink tissues unable to produce enough amounts of photosynthates themselves (Biemelt and Sonnewald, 2006).

At Montecillo (Figure 1A) at flowering, biomass of 'Seri' non-inoculated main stem was slightly higher than that of 'Rebeca' which means that in the absence of bacteria in normal cropping, 'Seri' MS is as vigorous as that of 'Rebeca' probably because 'Seri' maturity is longer than 'Rebeca' (Villaseñor and Espitia, 2000). It was also observed that bacteria produced a decrease on stems biomass in both cultivars at flowering, proportionally to the increase of inoculum on 'Seri', but not on 'Rebeca'. At maturity, MS biomass was greater than at flowering in both cultivars, but 'Rebeca' control plants had higher biomass increase than 'Seri'. Furthermore, at this sampling, the damage caused by the bacteria to stems was diluted, since the main stem weight of inoculated plants was similar to the control plants. At Chapingo (Figure 1F) (wetter climate, slightly cooler) 'Rebeca' produced heavier main stems than 'Seri' in both samplings, due to its better adaptation

**Table 5.** Mean squares and significance [1] of the sources of variation for dry weight of wheat organs measured in two samplings

| Variable | Cultivar (C) | | Dose (D) | | C × D | | CV (%) | |
|---|---|---|---|---|---|---|---|---|
| | Montecillo | Chapingo | Montecillo | Chapingo | Montecillo | Chapingo | Montecillo | Chapingo |
| *Main stems at flowering* | | | | | | | | |
| Stems | 0.03 ** | 0.12 ** | 0.08 ** | 0.03 ** | 0.01 * | 0.004ns | 12 | 11 |
| Sheaths | 0.04 ** | 0.17 ** | 0.002 ** | 0.05 ** | 0.0002 * | 0.02 ** | 5 | 3 |
| Laminae | 0.05 ** | 0.10 ** | 0.02 ** | 0.06 ** | 0.002ns | 0.02 ** | 15 | 11 |
| Spikes | 0.57 ** | 0.04 ** | 0.15 * | 1.53 ** | 0.02ns | 0.04 ** | 15 | 1 |
| Total per plant | 0.03ns | 0.78 ** | 0.72 ** | 3.04 ** | 0.04ns | 0.15 ** | 10 | 2 |
| *Main stems at maturity* | | | | | | | | |
| Stems | 0.10 ** | 0.25 ** | 0.01 ** | 0.02 ** | 0.002ns | 0.01 * | 6 | 9 |
| Sheaths | 0.03 ** | 0.01 ** | 0.002 ** | 0.02 ** | 0.0001 * | 0.01 ** | 5 | 6 |
| Laminae | 0.03 ** | 0.10 ** | 0.003 ** | 0.02 ** | 0.0001 * | 0.002 ** | 5 | 8 |
| Spikes | 0.01 ** | 0.48 ** | 1.44 ** | 0.13 * | 0.13 ** | 0.02ns | 1 | 15 |
| Total per plant | 1.12 ** | 0.001ns | 2.40 ** | 0.37 ** | 0.15 ** | 0.01ns | 3 | 9 |
| *Secondary stems at flowering* | | | | | | | | |
| Stems | 1.92 ** | 1.32 ** | 0.77 ** | 0.63 ** | 0.27 ** | 0.17 ** | 1 | 1 |
| Sheaths | 0.003ns | 0.01 * | 0.04 ** | 0.15 ** | 0.02 ** | 0.09 ** | 11 | 7 |
| Laminae | 0.17 ** | 0.10 ** | 0.28 ** | 0.15 ** | 0.03 ** | 0.03 ** | 5 | 7 |
| Spikes | 0.60ns | 0.83 ** | 1.14ns | 1.75 ** | 0.09ns | 0.33 ** | 23 | 6 |
| Total per plant | 6.90 ** | 0.21 ** | 6.86 ** | 8.25 ** | 0.72ns | 1.33 ** | 4 | 3 |
| *Secondary stems at maturity* | | | | | | | | |
| Stems | 0.02ns | 0.33 ** | 0.47 ** | 0.66 ** | 0.24 ** | 0.05 ** | 10 | 4 |
| Sheaths | 0.02 ** | 0.002 ** | 0.06 ** | 0.20 ** | 0.03 ** | 0.05 ** | 9 | 2 |
| Laminae | 0.16 ** | 0.05 ** | 0.18 ** | 0.13 ** | 0.01 * | 0.02 ** | 10 | 3 |
| Spikes | 1.89 ** | 0.14 ** | 0.86 * | 2.31 ** | 0.16ns | 0.31 ** | 21 | 6 |
| Total per plant | 4.20 ** | 0.14 ** | 5.03 ** | 9.65 ** | 1.10 ** | 0.99 ** | 10 | 2 |
| *Total per plant at flowering* | | | | | | | | |
| Stems | 2.41 ** | 2.24 ** | 1.29 ** | 0.88 ** | 0.19 ** | 0.20 ** | 4 | 4 |
| Sheaths | 0.18 * | 0.10 ** | 0.04ns | 0.36 ** | 0.03ns | 0.19 ** | 28 | 5 |
| Laminae | 0.38 ** | 0.39 ** | 0.46 ** | 0.39 ** | 0.05 ** | 0.08 ** | 4 | 6 |
| Spikes | 0.001ns | 1.21 ** | 2.10 * | 6.14 ** | 0.18ns | 0.19** | 17 | 3 |
| Total per plant | 6.86** | 1.76** | 10.95** | 21.03** | 0.38ns | 2.04** | 11 | 2 |
| *Total per plant at maturity* | | | | | | | | |
| Stems | 0.21* | 1.16** | 0.60** | 0.99** | 0.27** | 0.07** | 7 | 4 |
| Sheaths | 0.10** | 0.06** | 0.09** | 0.33** | 0.03** | 0.09** | 7 | 2 |
| Laminae | 1.58* | 0.17** | 0.43ns | 0.17** | 0.17ns | 0.02** | 55 | 2 |
| Spikes | 0.47ns | 0.09** | 1.67** | 6.82** | 0.12ns | 0.35** | 15 | 3 |
| Total per plant | 7.42** | 2.02** | 8.02** | 20.85** | 1.09ns | 1.11** | 12 | 2 |

[1] *, ** Significant at 5 and 1 %, respectively.

to the region, although the negative effect of the pathogen was similar in both cultivars without a clear dose effect. In both locations and cultivars, biomass gains of the main stems during seed filling were evident, particularly in inoculated plants, meaning that the stem functioned as an organ of demand during that period, especially in 'Seri'. At this stage, the mass of the stem does not include the spike nor the seed.

By a similar analysis, leaf sheaths (Figure 1B, 1G) and laminae (Figure 1C, 1H) of the MS of 'Rebeca ' accumulated more biomass in both locations and both samplings than that of 'Seri', in part because 'Rebeca' has larger leaves than 'Seri' since both have the same number of phytomers. However, in all situations both organs reduced biomass during the post-anthesis period, especially for the control plants of 'Rebeca' at Chapingo. Thus, sheaths and laminae functioned as a

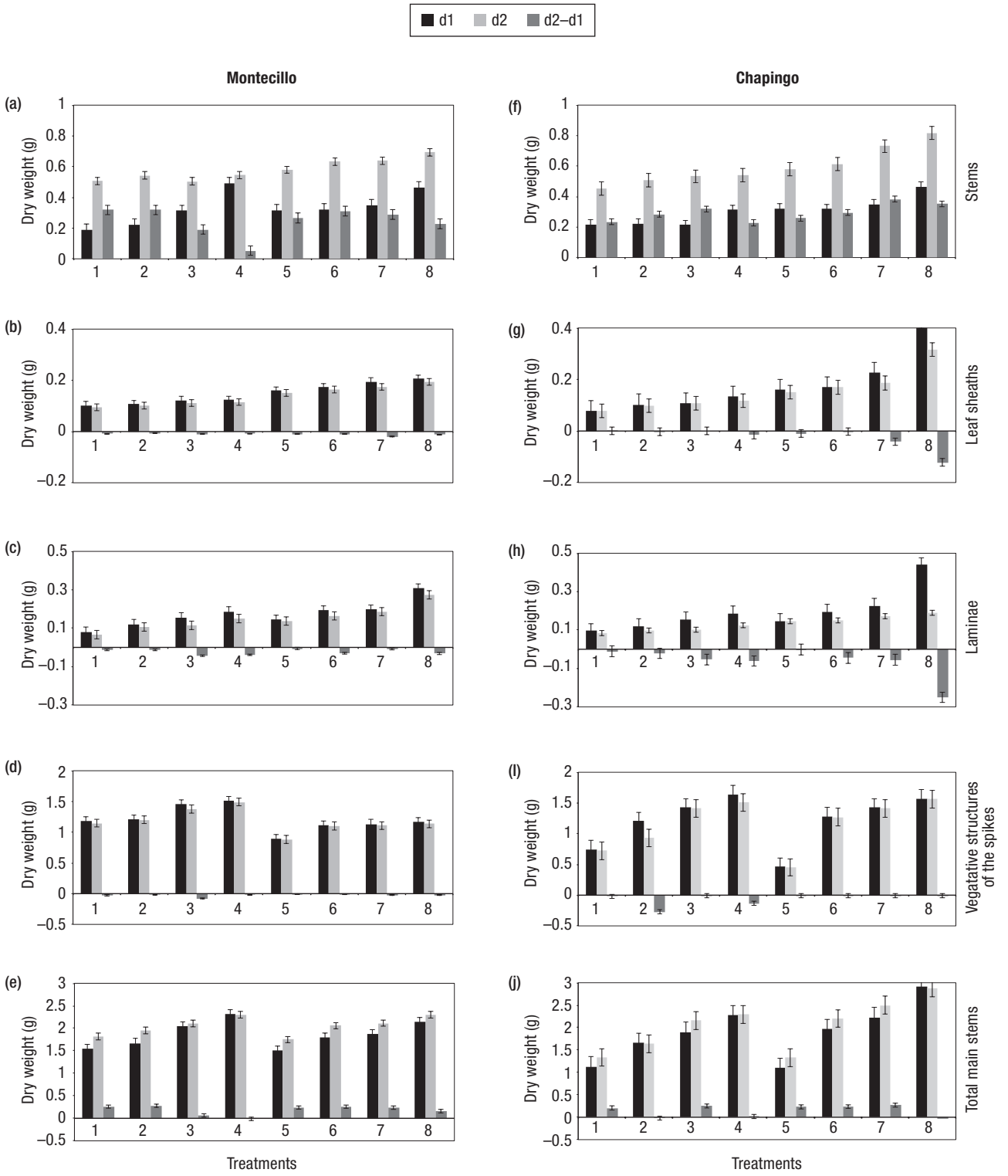


**Figure 1.** Dry weight at flowering (d1), at seed physiological maturity (d2), and difference with respect to flowering (d2-d1) of stems (a and f), leaf sheaths (b and g), lamina (c and h), vegetative structures of the spikes (d and i), and total of the main stems (e and j) of 'Seri M82' (Treatments 1 to 4) and 'Rebeca F2000' (Treatments 5 to 8), inoculated with three doses of *Pss* inoculum: $10^{10}$ (Treatments 1 and 5), $10^{8}$ (Treatments 2 and 6), $10^{6}$ (Treatments 3 and 7) cfu $mL^{-1}$, and control (Treatments 4 and 8), at Montecillo (left) and Chapingo (right). Error bars correspond to the standard error for each mean.

source even in the absence of the pathogen, which explains the compensation of carbohydrates from non-affected tissue during post-anthesis (Ziberstein *et al*., 1985; Valadez-Gutiérrez *et al*., 2006). Interestingly, in any condition and treatment, biomass accumulated in vegetative structures of 'Seri' spikes (Figure 1D, 1I) was greater than in 'Rebeca'. 'Seri' is a cultivar with high seed yield potential under favorable conditions in the northwest of México (States of Sinaloa and Sonora) and it was genetically derived from crosses between Veery's. In addition, because of the spike proximity to the seed (the main physiological sink during post-anthesis), it has been assumed that the vegetative components of the spike play an important role as source organ in this stage. Our data does not support this, since overall vegetative biomass of the spikes was similar in both samplings. Finally, the sum of biomass from main stem organs (Figure 1E, 1J) indicates that despite their lower seed yield, aerial biomass of vegetative structures of 'Seri' in both sampling and locations was similar to 'Rebeca' counterpart, except that at Chapingo control plants of 'Rebeca' accumulated more total biomass than control plants of 'Seri'. The negative effect of inoculum dose can be seen most clearly in this variable than any other.

For the secondary stems (data not shown), the largest magnitude of the values of some traits was due to tillers. However, trends in biomass accumulation were similar to those of the main stem, in that: (a) stems functioned as sink organs, and sheaths and limbs as source organs; and (b) the effect of inoculum doses was not clearly expressed in all organs as it was in the total biomass. In contrast to what occurred in the main stem, spikes of secondary stems of both cultivars at Montecillo, where *Pseudomonas* incidence was lower, performed as a source of assimilates during post-anthesis in all treatments, so total aerial biomass of secondary stems was lower at maturity than at flowering in this location.

Finally, trends in production and distribution of total plant biomass (sum of dry weight of main and secondary stems, data not shown) were similar to that of secondary stems. It was noted that total biomass of the vegetative organs of 'Seri' inoculated plants (susceptible to bacteria) at Chapingo, in both samplings was similar to respective treatments in 'Rebeca' (assumed tolerant). Therefore, it is confirmed that bacterial incidence and severity rankings do not necessarily match with the crop physiologic traits associated with the production and distribution of biomass (Valencia-Botín *et al*., 2007), including seed yield.

## Conclusions

*Pseudomonas* incidence and severity were higher at Chapingo than at Montecillo and in 'Seri M82' than in 'Rebeca F2000'. The yield and yield components were higher in 'Rebeca F2000' than in 'Seri M82', although total biomass production or that accumulated in the plant organs was generally similar in both cultivars. Inoculations of *Pss* reduced plant height, seed yield, and biomass accumulated in most organs of the main and secondary stems and total per plant. Overall, the magnitude of the reductions were similar in both cultivars; indicating that in effect 'Seri M82' is susceptible to this pathogen but the assumed tolerance of 'Rebeca F2000' is questioned. The inoculated pathogen affected seed number than seed size. The complexity of the sink-source relationships during post-anthesis was demonstrated in this study by the frequent significance of the cultivar × dose interaction in each location. However, stems prevailed as sink organs while laminae, sheaths and spikes served as source organs. The need to include physiologic traits in the evaluation of plant diseases caused by plant pathogenic bacteria is demonstrated.

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