Effect of Timing and Duration of Soil Saturation on Soilborne *Pythium* Diseases of Common Bean (*Phaseolus vulgaris*)

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Abstract

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Understanding combined abiotic (waterlogging) and biotic (*Pythium* spp.) stress resistance remains an important challenge to improving common bean (*Phaseolus vulgaris*) productivity in disease-prone regions with irregular but intensive rainfall patterns. This study documented the effects of timing (1, 3, 5, 7, and 9 days after sowing) and duration (3, 6, 12, and 24 h) of soil saturation (waterlogging) on damping-off, as well as hypocotyl and root diseases of common bean caused by *Pythium irregulare*. There were significant effects of timing of waterlogging as well as the presence or absence of the pathogen on emergence of the three bean varieties tested; namely, 'Gourmet Delight', 'Brown Beauty', and 'Pioneer'. The interaction between time of waterlogging and variety was significant for both root and hypocotyl disease severities. In the presence of *P. irregulare*, waterlogging 1 day after sowing resulted in the least emergence ($55.2 \pm 5.6\%$), although plants that survived after 5 weeks had less hypocotyl and root disease (percent hypocotyl disease index [%HDI] $\pm$ standard deviation [SD] = $42.0 \pm 2.1\%$ and percent root disease index [%RDI] $\pm$ SD = $42.4 \pm 2.1\%$, respectively) than nonwaterlogged plants (%HDI = $50.8 \pm 2.1\%$ and %RDI = $48.0 \pm 2.1\%$, respectively). The most severe disease assessed 5 weeks after sowing occurred when plants were subjected to waterlogging 9 days after sowing (%HDI = $61.3 \pm 2.1\%$ and %RDI = $56.0 \pm$

2.1%). In general, both hypocotyl and root disease severity increased as the duration of waterlogging increased from 1 to 24 h, with %HDI increasing from $53.9 \pm 3.2\%$ to $70.9 \pm 3.2\%$, while %RDI increased from $57.2 \pm 1.5\%$ to $73.7 \pm 1.5\%$. Varieties responded differentially in terms of disease development after waterlogging, with the least hypocotyl and root disease on Gourmet Delight (%HDI = 51.4 ± 3.2 and %RDI = 60.1 ± 1.5 , respectively) and greatest on Pioneer (%HDI = 66.2 ± 3.2 and %RDI = 64.9 ± 1.5 , respectively). Despite being susceptible to hypocotyl and root disease, Pioneer had the greatest emergence and shoot dry weight overall among the three varieties, suggesting that this variety has a degree of tolerance to waterlogging, *P. irregulare* infection, and the combination of these two stresses. Although the resistance of Gourmet Delight could be exploited to breed bean varieties that exhibit less hypocotyl and root disease when waterlogging occurs, the tolerance to both *P. irregulare* infection and waterlogging observed for Pioneer could also be exploited to breed varieties that incur less damage from hypocotyl or root disease or waterlogging. Furthermore, this study demonstrated what appears to be independent resistance to hypocotyl versus root infection by *P. irregulare*, which offers an opportunity to combine resistance to both stresses to reduce the impact of damping-off and root rot in conditions conducive for *P. irregulare*.

Common bean (*Phaseolus vulgaris*) is an economically important crop worldwide and an important grain legume for human consumption (13,30,33). However, diseases, including soilborne diseases, constitute a significant challenge to common bean production (33). *Pythium* spp. that can cause damping-off, hypocotyl, or root diseases on common bean have been reported frequently as a major cause of economic losses worldwide (1,17,20,30,37,40). In Western Australia, *Pythium* spp. are commonly associated with root disease or damping-off in legumes, with several *Pythium* spp. occurring widely on pasture legumes, (7,9) and common bean (44) (Y. P. Li, data not published.). In particular, *Pythium irregulare* is considered the most widespread and commonly occurring species in this region of Australia (8,10).

A range of disease symptoms from one or more *Pythium* spp. can occur on common bean, and include a seed rot below the soil surface or seedlings that do not survive long enough to emerge from the soil (preemergence damping-off) (1,38), seedlings that successfully emerge but with infected roots (root disease), and emerged seedlings that subsequently die from root or hypocotyl infection (postemergence damping-off) (1,38). For example, common bean seedling root infection by one or more *Pythium* spp. can develop rapidly, with roots showing brown discoloration and becoming necrotic, resulting in chlorotic symptoms on the foliage

and leading to plants wilting or dying as soon as a few weeks after sowing (38). Regardless of the type of symptoms, the consequent damage to common bean crops is generally evident as stand reduction from pre- or postemergence damping-off, or reduction in plant vigor of surviving plants from hypocotyl or root disease (24).

Environmental factors such as temperature and soil moisture can dramatically affect the severity of seedling hypocotyl or root disease on legumes such as pasture legumes (51) and common bean (34,39). In general, high soil moisture levels increase disease severity, particularly because *Pythium* spp. are favored by wet soil such as flooding conditions (18,39,52). Soil saturation from flooding, even brief durations of waterlogging, significantly promotes infection of roots by *Pythium* spp. and subsequent disease expression, because *Pythium* spp. thrive and produce massive numbers of motile zoospores under such conditions (22,35,52). Saturated soil conditions can greatly extend the size of hypocotyl lesions, even to the growing point of the young seedling, resulting in severe damping-off in common bean. Of the *Pythium* spp. known to infect common bean, *P. irregulare* is not only recognized as a major root pathogen but also causes softening of hypocotyl tissues at the soil surface (40). In both common bean and other legumes, *P. irregulare* can cause seedling collapse from severe root rot of emerged plants (40,53).

Soil saturation from waterlogging is also a constraint on plant growth and development, even in the absence of *Pythium* spp. or other plant pathogens, resulting in root damage and even plant death, particularly in situations of complete submergence from flooding (23). Soil waterlogging can cause severe yield losses for a range of legume crops, including soybean (19) and mung bean (48), particularly when flooding occurs at the crop reproductive growth stage. Such plant stress and consequent root damage and

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curtailing of plant growth provide *Pythium* spp. with an advantage for infecting plants in comparison with plants grown in well-drained soils without waterlogging, for common bean (39) and other legumes such as pea (27) and forage legumes (51). Increased susceptibility of plants to *Pythium* spp. can result from waterlogging because leakage of solutes from waterlogged roots stimulates spore germination of *Pythium* spp., thereby increasing disease incidence and severity (3,14,27,41).

Waterlogging–soilborne pathogen interactions remain poorly understood for common bean and many other crops. For example, in common bean, no variety has been identified with resistance to both waterlogging stress and *Pythium* infection. Understanding combined resistance to this abiotic stress and *Pythium* infection is currently one of the main challenges to improve common bean production in regions with irregular rainfall patterns (2), and is expected to be even more important under increased erratic rainfall patterns predicted as a result of climate change (25,31). Developing stress-resistant varieties is a primary target of plant breeders, because deploying resistant varieties is one of the most cost-effective ways to manage challenges from abiotic or biotic stresses of common bean (33) and other crops (6,25).

This study examines the role of soil saturation (waterlogging) on the severity of soilborne diseases of common bean caused by *P. irregulare*, and demonstrates the impact of soil saturation timing (1, 3, 5, 7, and 9 days after sowing) and duration (3, 6, 12, and 24 h) on damping-off and hypocotyl and root infection of common bean caused by *P. irregulare*. The study also examines differences among three common bean varieties in resistance (the ability of a plant to reduce the extent of invasion by the pathogen; 26) or tolerance (the ability of a plant to continue to grow and yield despite a certain level of infection; 26) to *P. irregulare* and soil saturation, and opportunities to develop varieties that are more resilient and productive for regions prone to these stresses.

Materials and Methods

Bean varieties. Three commercial Australian common bean varieties (namely, ‘Gourmet Delight’, ‘Brown Beauty’, and ‘Pioneer’; Yates Seed Co., Padstow, NSW, Australia) were used in this study. Seed was sourced commercially and not treated with fungicides.

Pathogen isolate. *P. irregulare* isolate WAC4953 was used in this study because the isolate had been used extensively in research on seedling damping-off and root rot on different legumes in Western Australia (53), and is representative of the prevailing *P. irregulare* populations on crop and forage legumes in Western Australia (M. J. Barbetti, data not published.).

Experimental conditions. Two experiments were undertaken: a waterlogging timing experiment and a waterlogging duration experiment. For both experiments, bean seed was sown 5 cm deep in free-draining pots (2 seeds/pot [6 by 6 by 10 cm]) filled with University of Western Australia (UWA) potting mix (finely crushed pine bark/coco peat/sand at 2.5:1.0:1.5 [wt/wt]). The potting mix was pasteurized for 90 min at 65°C before use. All plants were grown at 18°C and with a 12-h photoperiod/day. In cases where both seedlings in a pot emerged, the mean values for both seedlings were determined and recorded as a single value. Both experiments were repeated.

Inoculum. *P. irregulare* was cultured on potato dextrose agar (PDA) for 5 days at 25°C in the dark until mycelium had almost grown across the plate. Then, PDA plugs (1 by 1 cm) from the leading edge of a colony were cut and mixed with sterilized millet seed to produce inoculum. Millet seed were prepared by first soaking the seed in deionized water (40 g of millet seed soaked in 100 ml of water to make 150 ml of millet seed-water mixture/250-ml flask) overnight, draining the excess water, and autoclaving the moist millet seed at 121°C for 20 min, once on each of three consecutive days. The millet seed and *P. irregular*-colonized agar pieces were then incubated at 25°C in the dark for 2 weeks. Flasks were shaken by hand once a day the first 2 days to ensure that PDA plugs were evenly distributed among the seed. At the time of in-

oculation of the potting medium, quality of the inoculum was checked by plating approximately 15 colonized millet seeds onto corn meal agar (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) plates to confirm that *P. irregulare* was the sole organism present on the seed. Colonized millet seed were mixed with pasteurized UWA potting mix at 0.5% (wt/wt) immediately prior to sowing bean seed. In each experiment, 200 g of inoculated soil was placed in each pot, and pots were then watered daily.

Waterlogging treatments. Timing of waterlogging. In the waterlogging timing experiment, five timings of initiating soil saturation by waterlogging plus a nonwaterlogged treatment that was near water-holding capacity were tested for the effects on bean growth and disease caused by *P. irregulare*. Waterlogging treatments were each for a 3-h duration and initiated 1, 3, 5, 7, or 9 days after bean seed had been sown. To implement waterlogging, each pot was placed in a large plastic container and water was added to the plastic container to the level of the surface of the potting mix in the pot to ensure that the potting mix medium in the pot was fully saturated.

Duration of waterlogging. For the waterlogging duration experiment, six durations of waterlogging were investigated. Three days after sowing, pots were saturated as described above for 0, 3, 6, 12, 24, or 48 h. The water level in the plastic container in which each pot was placed was maintained to the top of the potting mix in the pot until the pot was removed from the large container at the end of each waterlogging duration.

Disease and dry weight assessments, and calculation of the tolerance of bean varieties to hypocotyl or root disease and waterlogging. Seedling emergence was recorded 10 days after sowing. Disease assessment of emerged seedlings was made 5 weeks after sowing. Seedlings were cut at the soil surface and the roots and shoots were separated. Roots were washed thoroughly with pressurized water. Then, the severity of hypocotyl and root disease was assessed using a visual disease assessment scale modified from that of Abawi and Pastor-Carrales (1). Hypocotyl disease severity was measured as follows: 0 = no apparent hypocotyl symptoms, 1 = maximum 10% of hypocotyl surface area with lesions, 2 = >10 to 25% of hypocotyl with lesions, 3 = >25 to 50% of hypocotyl with lesions, 4 = >50 to 75% of hypocotyl with lesions, and 5 = >75% of hypocotyl with lesions or the plant had died. Root disease severity was measured as follows: 0 = no apparent root symptoms, 1 = <20% of roots showing obvious lesions, 2 = root system showing a moderate level of decay, 3 = root system showing extensive decay with an obvious reduction in overall root system size (<50% of root system missing), and 4 = root system with very severe decay and extensive reduction in overall root size (≥50% root system missing) or the plant had died. Shoot material for each plant was oven dried at 60°C for 3 days, and dry weight was recorded. Root dry weights were also recorded similarly and used to calculate the level of tolerance of the three bean varieties for each of the five timings of soil saturation and each of the six durations of waterlogging by expressing mean dry root weight of the waterlogged plants, with or without inoculation with *P. irregulare*, as a fraction of the respective nonwaterlogged plants.

Experimental design and statistical analyses. A completely randomized experimental design with a five-by-two factorial treatment design of waterlogging timing treatment–inoculation treatment was arranged for the experiment on timing of waterlogging and the repeat of this experiment, with eight replications used per experiment and two seeds sown per pot for each treatment combination. For the experiment on duration of waterlogging and the repeat of this experiment, six replications were used per experiment, with two seeds sown per pot for each treatment combination, arranged in a completely randomized experimental design with a six-by-two factorial treatment design of waterlogging duration–inoculation treatment.

For hypocotyl and root disease score data, percent disease index (%DI) was calculated using the formula of McKinney (32), where $\%DI = \{[(a \times 0) + (b \times 1) + (c \times 2) + (d \times 3) + (e \times 4) + (f \times 5)] / [(a + b + c + d + e + f) \times g]\} \times 100$, where *a*, *b*, *c*, *d*, and *e* represent

the number of plants with disease scores of 0, 1, 2, 3, and 4, respectively, for root ratings; *a*, *b*, *c*, *d*, *e*, and *f* represent hypocotyl ratings of 0, 1, 2, 3, 4, and 5, respectively; and *g* represents the highest rating of root disease or hypocotyl disease. Percent emergence, hypocotyl disease severity, root disease severity, and shoot dry weight data were analyzed separately for the two waterlogging experiments using analysis of variance with GenStat (15th ed.; Lawes Agricultural Trust, Hemel, Hempstead, United Kingdom). Fisher's least significant difference ($P < 0.05$) was used to compare variety ratings. All the P values obtained from GenStat were then further recalculated to four decimal points (43).

Results

The relationship between the two repeats of the timing of waterlogging experiment and between the two repeats of the duration of waterlogging experiment were assessed both by using a one-sided paired t test and by regression analyses for each disease parameter. These tests showed no significant differences for pairs of repeated experiments ($P > 0.05$) and significant correlations between data sets (e.g., the correlation coefficient [r] of root disease index for the two timing of waterlogging experiments was 0.924 with $P < 0.0001$, and between the two duration of waterlogging experiments was 0.922 with $P < 0.0001$). The Brown-Forsythe test (15) was also conducted to determine whether variances between data sets of repeat experiments were homogeneous. Because variances of the repeat data sets were homogeneous and the interactions between treatments and experiments were not significant ($P > 0.05$) for all dependent variables in each experiment, data were pooled and reanalyzed as a single data set for each pair of the timing and duration waterlogging experiments.

Timing of waterlogging. *Seedling emergence.* Seedling emergence was affected significantly by the main effects of inoculum ($P < 0.0001$) and timing of waterlogging ($P < 0.0001$). There was also a significant interaction between timing of waterlogging with inoculum ($P = 0.0063$) and timing of waterlogging with variety ($P = 0.0136$) (Table 1). On average, plants emerged 6 to 7 days after sowing.

Based on the means averaged over all three bean varieties, waterlogging did not affect seedling emergence in the noninoculated pots, with emergence ranging from 89.6 to 97.9% (Table 2). In contrast, in the inoculated pots, very early waterlogging reduced seedling emergence the greatest. Pots waterlogged 1 day after sowing had the least emergence ($55.2 \pm 5.6\%$), and emergence was also reduced significantly in pots waterlogged 3 and 5 days after sowing. At 7 or 9 days after sowing, no significant reduction in emergence from waterlogging was observed.

Disease severity. Disease severity on hypocotyls (percent hypocotyl disease index [%HDI]) and roots (percent root disease index [%RDI]) was affected significantly by the main effects of inoculum

($P < 0.0001$ for both variables), variety ($P < 0.0001$ for both variables), and timing of waterlogging ($P < 0.0001$ for both variables). There was also a significant interaction between inoculum and variety ($P < 0.0001$ for both variables) and between inoculum and timing of waterlogging ($P < 0.0001$ for both variables) (Table 1). There were no hypocotyl or root disease symptoms in any of the noninoculated pots for all three bean varieties (Table 2). Based on the overall means averaged for all three varieties and for all inoculated plants, waterlogging increased disease severity on both hypocotyls and roots, except in the pots exposed to the earliest waterlogging, 1 day after sowing (Table 2). For pots waterlogged 1 day after sowing for 3 h, hypocotyl and root disease severity was significantly less than that of the nonwaterlogged pots. However, in general, hypocotyl and root disease both increased as the timing of initiation of waterlogging was delayed to 3 days or later after sowing. The most severe hypocotyl infection was observed when pots were waterlogged 5 to 9 days after sowing (%HDI reached 61.3 ± 2.1), and the most severe root symptoms occurred in pots waterlogged 3 to 9 days after sowing (%RDI reached 56.0 ± 2.1). There was no significant difference in root disease severity when waterlogging occurred ≥ 3 days after sowing, except pots waterlogged 7 days after sowing, when a slight reduction in root disease severity was observed compared with 5 and 9 days but there was still more severe root rot than nonwaterlogged plants. There was no significant difference in the severity of hypocotyl symptoms when waterlogging occurred ≥ 5 days after sowing. The three bean varieties responded differently to infection by *P. irregulare* after waterlogging. Gourmet Delight had the least severe symptoms on hypocotyls and roots, followed by Brown Beauty, and Pioneer had the most severe symptoms (Table 2).

Shoot dry weight. Shoot dry weight was affected significantly by the main effects of inoculum ($P < 0.0001$), variety ($P < 0.0001$), and timing of waterlogging ($P = 0.0008$). There was also a significant interaction between inoculum and variety ($P = 0.0084$) and between inoculum and timing of waterlogging ($P = 0.0341$) (Table 1). Based on the means averaged over the three varieties, shoot dry weight of plants was greater in noninoculated pots than inoculated pots for both nonwaterlogged and waterlogged treatments; this was also true for all three bean varieties (Table 2). Shoot dry weight was not affected consistently by initial waterlogging timing. In the noninoculated pots, the greatest shoot dry weight was recorded when pots were waterlogged 1, 3, 7, or 9 days after sowing (480.5 to 519.8 mg/plant) but waterlogging 5 days after sowing resulted in a reduction in shoot dry weight (437.9 ± 30.3 mg/plant) compared with other waterlogging timings but not compared with nonwaterlogged plants. There was no significant difference in shoot dry weight in pots not waterlogged versus waterlogged 1, 7, or 9 days after sowing. In the inoculated pots, a reduction in shoot dry weight was observed when pots were waterlogged 1 day after sow-

Table 1. Statistical main effects and interactions for the analysis of variance of timing of waterlogging experiments on emergence, hypocotyl disease index, root disease index, and shoot dry weight of three varieties of common bean (*Phaseolus vulgaris*) inoculated or not inoculated with *Pythium irregulare*^a

Main effects, interactions ^a	Emergence (%)		Hypocotyl disease index (%)		Root disease index (%)		Shoot dry weight (mg/plant)	
	<i>P</i> value	LSD	<i>P</i> value	LSD	<i>P</i> value	LSD	<i>P</i> value	LSD
Inoculum (Inoc)	<0.0001	4.5	<0.0001	1.9	<0.0001	1.7	<0.0001	24.3
Variety (Var)	0.0908	n.s.	<0.0001	2.3	<0.0001	2.0	<0.0001	29.8
Waterlogging time (Time)	<0.0001	7.9	<0.0001	3.2	<0.0001	2.9	0.0008	42.1
Inoc × Var	0.3874	n.s.	<0.0001	3.2	<0.0001	2.9	0.0084	42.1
Inoc × Time	0.0063	11.1	<0.0001	4.5	<0.0001	4.0	0.0341	59.5
Var × Time	0.0136	13.6	0.7618	n.s.	0.2272	n.s.	0.0928	n.s.
Inoc × Var × Time	0.6288	n.s.	0.7618	n.s.	0.2272	n.s.	0.1858	n.s.

^a Each experiment was arranged in a completely randomized design and repeated once with eight replicate pots/treatment combination/experiment, and two bean seeds planted per pot for each replication. Means within a column followed by the same letter do not differ significantly based on Fisher's least significant difference (LSD) at $P = 0.05$; n.s. = not significant at $P = 0.05$. Emergence was recorded 10 days after sowing; hypocotyl disease index and root disease index were recorded 5 weeks after sowing and disease indices calculated as described in the main text; shoots were placed in an oven at 60°C for 3 days after harvesting and dry weights were recorded. Results were pooled over the repeats of the experiment as described in the main text.

^b Inoculum = pots were inoculated or not inoculated with *P. irregulare*. Three bean varieties were used: Gourmet Delight, Brown Beauty, and Pioneer, as described in the main text. Pots were each waterlogged for 3 h at 1, 3, 5, 7, or 9 days after sowing.

ing for two varieties, Gourmet Delight and Brown Beauty, but not for Pioneer (Table 2). However, shoot dry weight increased when waterlogging was applied 7 or 9 days after sowing. Shoot dry weight differed among the three bean varieties when not inoculated with *P. irregulare*, with Pioneer having the greatest shoot dry weight, followed by Brown Beauty and Gourmet Delight. However, for inoculated plants, there was no significant difference in shoot dry weight between the latter two, whereas Pioneer had the greatest shoot dry weight (Table 2).

Duration of waterlogging. *Seedling emergence.* Seedling emergence was affected significantly by the main effects of inoculum ($P < 0.0001$) and duration of waterlogging ($P < 0.0001$), and there was a significant interaction between inoculum and variety ($P = 0.0381$) (Table 3). For both noninoculated and inoculated pots, no seedlings emerged for the first 48 h of waterlogging after sowing. Based on the means averaged over the three bean varieties, seedling emergence generally declined as the duration of waterlogging increased (Table 4). Noninoculated pots of Pioneer had more plants emerged than inoculated pots for both nonwaterlogged and waterlogged treatments but not for Brown Beauty and Gourmet Delight in nonwaterlogged pots.

Disease severity. Hypocotyl disease severity was affected significantly by the main effects of inoculum ($P = 0$), variety ($P < 0.0001$), and duration of waterlogging ($P < 0.0001$) (Table 3). There were also significant interactions between inoculum and variety ($P < 0.0001$), inoculum and duration of waterlogging ($P < 0.0001$), and variety and duration of waterlogging ($P = 0.0099$). Additionally, there was a significant three-way interaction ($P =$

0.0099) between inoculum, variety, and waterlogging duration (Table 3). Root disease severity similarly was affected significantly by the main effects of inoculum ($P = 0$), variety ($P = 0.0475$), and duration of waterlogging ($P < 0.0001$); the interactions between inoculum and variety ($P = 0.0475$), inoculum and duration of waterlogging ($P < 0.0001$), and variety and duration of waterlogging ($P = 0.0001$); and the three-way interaction between inoculum, variety, and duration of waterlogging ($P = 0.0001$) (Table 3). Based on the overall mean severity ratings for inoculated plants of all three varieties, waterlogging increased both hypocotyl and root disease severity (Table 4). Plants showed the most severe hypocotyl symptoms when pots were waterlogged for 3, 6, or 12 h (%HDI = 62.3 ± 2.0 , 70.9 ± 2.0 , and 59.8 ± 2.0 , respectively), and showed the most severe root symptoms when pots were waterlogged for 12 h (%RDI = 73.7 ± 1.9). Hypocotyl disease severity declined when pots were waterlogged for 24 h (%HDI = 59.8 ± 2.0). However, the three bean varieties responded differently to duration of waterlogging. Gourmet Delight had the least severe disease on both hypocotyls (%HDI averaged 51.4 ± 2.4) and roots (%RDI averaged 60.1 ± 2.4), whereas Pioneer and Brown Beauty had the most severe disease on hypocotyls (%HDI averaged 66.2 ± 2.4 and 65.3 ± 2.4 , respectively) and roots (%RDI averaged 64.9 ± 2.4 and 64.7 ± 2.4 , respectively), with no difference between these varieties for either variable. Gourmet Delight and Brown Beauty showed the most severe root symptoms when waterlogging was applied for 12 h, whereas Pioneer showed the most severe root disease after waterlogging for 24 h. For Brown Beauty and Pioneer, the most severe hypocotyl symptoms were observed after pots were waterlogged

Table 2. Effect of timing of waterlogging on emergence, hypocotyl disease index (HDI), root disease index (RDI), and shoot dry weight (SDW) of three varieties of common bean (*Phaseolus vulgaris*; Gourmet Delight, Brown Beauty, and Pioneer) inoculated (Inoc) or not inoculated (Noninoc) with *Pythium irregulare*^x

Variable, WT ^z	Gourmet Delight		Brown Beauty		Pioneer		Overall mean of WT ^y	
	Inoc	Noninoc	Inoc	Noninoc	Inoc	Noninoc	Inoc	Noninoc
Emergence (%)								
Nonwaterlogged	93.7 e	97.0 b	75.0 c	90.6 bc	93.8 c	100.0 b	87.5 c	95.8 a
1 DAS	50.0 a	97.0 b	46.9 a	81.2 a	68.8 a	90.6 a	55.2 a	89.6 a
3 DAS	84.4 d	100.0 b	65.6 b	87.5 ab	81.2 b	100.0 b	77.1 b	95.8 a
5 DAS	62.5 b	84.4 a	78.1 c	96.9 cd	90.6 c	90.6 a	77.1 b	90.6 a
7 DAS	71.9 c	97.0 b	93.8 d	96.9 cd	81.2 b	100.0 b	82.3 bc	97.9 a
9 DAS	87.5 de	93.8 b	96.9 d	100.0 d	87.5 bc	93.8 ab	90.6 c	95.8 a
Mean	75.0	94.8	76.0	92.2	83.9	95.8	78.3	94.3
%HDI								
Nonwaterlogged	49.4 b	0	46.7 b	0	56.3 b	0	50.8 b	0 a
1 DAS	35.0 a	0	41.0 a	0	50.0 a	0	42.0 a	0 a
3 DAS	53.1 cd	0	53.9 c	0	65.3 cd	0	57.4 c	0 a
5 DAS	52.3 bc	0	65.3 e	0	64.0 c	0	60.6 d	0 a
7 DAS	52.0 bc	0	61.5 d	0	64.4 c	0	59.3 cd	0 a
9 DAS	55.6 d	0	60.0 d	0	68.0 d	0	61.3 d	0 a
Mean	49.6	0	54.7	0	61.4	0	55.2	0
%RDI								
Nonwaterlogged	48.4 bc	0	44.2 a	0	51.6 b	0	48.0 b	0 a
1 DAS	36.5 a	0	45.8 a	0	45.0 a	0	42.4 a	0 a
3 DAS	52.3 d	0	51.9 b	0	60.0 d	0	54.2 cd	0 a
5 DAS	48.0 bc	0	60.0 c	0	59.0 d	0	55.8 d	0 a
7 DAS	46.6 b	0	53.8 b	0	55.0 c	0	52.0 c	0 a
9 DAS	50.0 cd	0	52.3 b	0	67.2 e	0	56.0 d	0 a
Mean	47.0	0	51.4	0	56.0	0	51.5	0
SDW (mg/plant)								
Nonwaterlogged	253.0 b	344.7 b	260.0 bc	485.9 c	431.5 a	583.0 a	314.9 b	471.2 ab
1 DAS	194.0 a	378.0 bc	146.8 a	440.0 bc	450.4 a	623.0 ab	265.8 a	480.5 bc
3 DAS	291.0 bc	391.0 c	236.6 b	386.6 a	465.5 a	781.0 e	331.0 b	519.8 c
5 DAS	252.0 b	301.0 a	293.1 cd	360.8 a	443.6 a	651.4 bc	329.7 b	437.9 a
7 DAS	297.0 c	392.0 c	315.8 d	430.3 b	511.6 b	691.0 cd	374.9 c	504.6 bc
9 DAS	284.0 bc	343.0 b	323.9 d	436.6 b	596.2 c	702.0 d	401.7 c	494.4 bc
Mean	262.0	358.6	262.7	423.4	484.0	672.2	336.2	484.7

^x Each experiment was arranged in a completely randomized design and repeated once with eight replicate pots/treatment combination/experiment, and two bean seeds planted/pot. Means within a column followed by the same letter do not differ significantly based on Fisher's least significant difference (LSD) at $P = 0.05$. Results were pooled over the repeats of the experiment as described in the main text.

^y WT = waterlogging timing after sowing bean seed.

^z DAS = days after sowing. All pots subjected to waterlogging were inoculated or not inoculated with *P. irregulare* prior to sowing, as described in the main text. %HDI and %RDI were calculated as explained in the main text.

for 6 h; for Gourmet Delight, this was true for both 3 and 6 h of waterlogging (Table 4).

Shoot dry weight. Shoot dry weight was affected significantly by the main effects of inoculum ($P < 0.0001$), variety ($P < 0.0001$), and duration of waterlogging ($P < 0.0001$). There were also significant interactions between inoculum and variety ($P < 0.0001$) and between inoculum and duration of waterlogging ($P < 0.0001$) (Table 3). Based on the overall means for all three varieties, shoot dry weight generally declined as the duration of waterlogging increased in both inoculated and noninoculated pots, with a few exceptions (Table 4). For noninoculated plants, as little as 3 h of waterlogging significantly reduced shoot dry weight compared with

nonwaterlogged control plants of all three varieties, with the smallest shoot dry weight detected when waterlogging was applied for 24 h (276.9 ± 34.9 mg/plant), except for Pioneer, which had the smallest shoots when waterlogged for 3 or 24 h in noninoculated pots. In the inoculated pots, shoot dry weight of plants was less than that of the noninoculated pots for both waterlogged and nonwaterlogged treatments. Varieties differed in terms of effects of treatments on shoot dry weight, with Pioneer having the greatest shoot dry weight in both noninoculated and inoculated plants compared with the other two varieties. There was no significant difference in shoot dry weight between Gourmet Delight and Brown Beauty in both noninoculated and inoculated pots.

Table 3. Statistical main effects and interaction terms for the analysis of variance of waterlogging duration (Duration) experiment for emergence, hypocotyl disease index, root disease index, and shoot dry weight of three varieties of common bean (*Phaseolus vulgaris*) inoculated or not inoculated with *Pythium irregulare*^y

Main effects, interactions ^z	Emergence (%)		Hypocotyl disease index (%)		Root disease index (%)		Shoot dry weight (mg/plant)	
	P value	LSD	P value	LSD	P value	LSD	P value	LSD
Inoculum (Inoc)	<0.0001	6.7	<0.0001	1.8	<0.0001	1.8	<0.0001	30.8
Variety (Var)	0.2198	n.s.	<0.0001	2.2	0.0475	2.1	<0.0001	37.7
Duration	<0.0001	10.6	<0.0001	2.8	<0.0001	2.8	<0.0001	48.7
Inoc × Var	0.0381	11.6	<0.0001	3.0	0.0475	3.0	<0.0001	53.3
Inoc × Duration	0.0618	n.s.	<0.0001	3.9	<0.0001	3.9	<0.0001	68.8
Var × Duration	0.1702	n.s.	0.0099	4.8	0.0001	4.8	0.2179	n.s.
Inoc × Var × Duration	0.3926	n.s.	0.0099	6.8	0.0001	6.7	0.8338	n.s.

^y Each experiment was arranged in a completely randomized design and repeated once with six replicate pots/treatment combination (two bean seeds/pot) planted. Means within a column followed by the same letter do not differ significantly based on Fisher's least significant difference (LSD) at $P = 0.05$; n.s. = not significant at $P = 0.05$. Emergence was recorded 10 days after sowing; hypocotyl disease index and root disease index were recorded 5 weeks after sowing and disease indices calculated as described in the main text; shoots were placed in an oven at 60°C for 3 days after harvesting and dry weights recorded. Results were pooled over the repeats of the experiment, as described in the main text.

^z Inoculum = pots were inoculated or not inoculated with *Pythium irregulare*. Three bean varieties were used: Gourmet Delight, Brown Beauty, and Pioneer, as described in the main text. Pots were waterlogged for 0, 3, 6, 12, 24, or 48 h at 3 days after sowing.

Table 4. Effect of waterlogging duration (duration) on emergence, hypocotyl disease index (HDI), root disease index (RDI), and shoot dry weight (SDW) of each of three varieties of common bean (*Phaseolus vulgaris*; Gourmet Delight, Brown Beauty, and Pioneer) inoculated (Inoc) or not inoculated (Noninoc) with *Pythium irregulare*^x

Variable, duration ^z	Gourmet Delight		Brown Beauty		Pioneer		Overall mean of WD ^y	
	Inoc	Noninoc	Inoc	Noninoc	Inoc	Noninoc	Inoc	Noninoc
Emergence (%)								
Nonwaterlogged	100.0 d	95.8 c	91.7 d	91.7 bc	70.8 c	100.0 b	87.5 d	95.8 c
3 h	79.2 c	95.8 c	66.7 bc	95.8 c	75.0 c	95.8 b	73.6 c	87.5 bc
6 h	66.7 b	83.3 b	75.0 c	83.3 b	70.8 c	91.7 b	70.8 c	80.6 b
12 h	20.8 a	83.3 b	62.5 b	83.3 b	54.2 b	91.7 b	45.8 b	80.6 b
24 h	29.2 a	54.2 a	37.5 a	54.2 a	37.5 a	70.8 a	34.7 a	61.1 a
Mean	59.2	82.5	66.7	70.8	61.7	90.0	62.5	81.1
%HDI								
Nonwaterlogged	42.5 a	0	63.3 a	0	56.0 a	0 a	53.9 a	0 a
3 h	58.3 c	0	64.6 a	0	64.0 c	0 a	62.3 c	0 a
6 h	58.9 c	0	72.5 b	0	78.9 e	0 a	70.9 d	0 a
12 h	55.0 b	0	63.3 a	0	61.0 b	0 a	59.8 bc	0 a
24 h	42.4 a	0	62.8 a	0	71.3 d	0 a	58.9 b	0 a
Mean	51.4	0	65.3	0	66.2	0 a	61.0	0
%RDI								
Nonwaterlogged	53.1 b	0	64.6 c	0	53.8 a	0 a	57.2 a	0 a
3 h	57.3 c	0	57.9 a	0	63.7 b	0 a	59.7 b	0 a
6 h	61.1 d	0	66.7 c	0	65.2 b	0 a	64.4 c	0 a
12 h	79.2 e	0	73.6 d	0	68.2 c	0 a	73.7 d	0 a
24 h	50.0 a	0	60.7 b	0	73.4 d	0 a	61.4 b	0 a
Mean	60.1	0	64.7	0	64.9	0 a	63.2	0
SDW (mg/plant)								
Nonwaterlogged	191.2 d	510.5 d	161.0 b	514.6 d	219.0 b	859.6 c	190.3 c	628.2 c
3 h	154.6 cd	305.8 c	183.4 b	380.8 c	166.0 a	512.1 a	168.0 bc	399.5 b
6 h	113.5 bc	255.5 b	138.3 ab	389.3 c	186.2 ab	602.4 b	146.1 abc	415.7 b
12 h	67.1 ab	291.0 bc	109.0 a	284.1 b	217.1 b	583.0 b	131.0 ab	385.8 b
24 h	42.8 a	145.8 a	108.0 a	196.2 a	156.0 a	489.3 a	101.9 a	276.9 a
Mean	113.8	301.7	139.9	353.0	189.1	609.0	147.6	421.2

^x Each experiment was arranged in a completely randomized design and repeated once with six replicate pots/treatment combination /experiment (two bean seeds planted/pot). Means within a column followed by the same letter do not differ significantly based on Fisher's least significant difference (LSD) at $P = 0.05$. Results were pooled over the repeats of the experiment as described in the main text. Data of 48 h were not included in the analysis because no seedlings emerged after waterlogging for 48 h.

^y WD = waterlogging duration. All pots subjected to waterlogging were inoculated or not inoculated with *P. irregulare* prior to sowing, as described in the main text.

^z %HDI and %RDI were calculated as explained in the main text.

Tolerance to waterlogging, and hypocotyl and root infection.

The level of tolerance to waterlogging expressed was affected significantly by variety ($P = 0.0150$) in the timing of waterlogging experiment and by variety ($P = 0.0170$) in the duration of waterlogging experiment. When the level of tolerance across the three varieties was calculated for each of the five timings of initiating waterlogging and each of the six durations of waterlogging, by expressing dry root weight of the waterlogged plants as a fraction of the respective nonwaterlogged plants, Pioneer showed the greatest tolerance. The mean tolerance for Pioneer was 1.20 ± 0.13 in the timing of waterlogging experiment and 1.09 ± 0.09 in the duration of waterlogging experiment (*data not shown*). In contrast, tolerance for Gourmet Delight and Brown Beauty were always <1 for both the timing of waterlogging and the duration of waterlogging experiments. In the timing of waterlogging experiment, the mean tolerance for Gourmet Delight was 0.92 ± 0.13 and for Brown Beauty was 0.80 ± 0.13 . In the duration of waterlogging experiment, the mean tolerance for Gourmet Delight was 0.91 ± 0.09 and for Brown Beauty was 0.81 ± 0.09 .

Discussion

Both the time of initiating waterlogging and the duration of waterlogging significantly affected the reactions of three bean varieties to *P. irregulare* and the interaction between plant growth and disease symptoms caused by *P. irregulare*. The results from this study concur with previous studies demonstrating that soil moisture can significantly affect the severity of root disease caused by *Pythium* spp. (12,27,34,39). Specifically, this study showed that even short durations of soil saturation can increase disease severity caused by *P. irregulare*. This was expected because *Pythium* spp. are favored by wet soil conditions (18,22,35,39,52). It is also possible that *P. irregulare* infection was promoted as a consequence of root damage and associated solute leakage caused by waterlogging, as well as stimulating spore germination of *P. irregulare* (3,14,27,41). Even a very brief duration of waterlogging (3 h) significantly promoted infection and consequent disease from *P. irregulare*. Waterlogging reduced seedling emergence, particularly when plants were waterlogged 1 day after sowing, as anticipated because *P. irregulare* can reduce emergence of legumes such as subterranean clover (*Trifolium subterraneum*), even without waterlogging (7).

Emergence of bean seedlings in this study decreased when waterlogging duration was increased, even in the absence of *P. irregulare*. Similar effects have been reported on other crops. For example, Hsu et al. (21) found that the emergence rate of Sudan grass (*Sorghum sudanense*) decreased significantly as the waterlogging duration increased. It is likely that the reason for decline in hypocotyl and root disease when waterlogging duration was ≥ 24 h in this bean study was because susceptible seed, whether germinating or not, were killed prior to emergence. Similarly, Yanar et al. (52) demonstrated that the longer saturated soil conditions lasted, the greater the negative effect on maize (*Zea mays*) plants due to the additive adverse interaction between *P. arrhenomanes* and excessive soil water on plant growth. In this bean study, shoot dry weight decreased as waterlogging duration increased, in part due to an adverse response of the host plant to waterlogging, and presumably, also, as a consequence of disease severity increasing with increasing waterlogging duration. Waterlogging, particularly for longer periods than evaluated in this study, has been shown to have a negative effect on plant growth and yield for many plant species; for example, 42 days of waterlogging for wheat, barley, and oat (50); 7 days of waterlogging for grain legumes (47); 7 days of waterlogging for soybean (29); and an unspecified duration of waterlogging for common bean (45). That *P. irregulare* played a significant role in shoot weight reduction in this study was anticipated because studies involving *P. irregulare* on other legumes have demonstrated a strong negative correlation between root disease severity and shoot dry weight (5,7,51).

The most severe root rot symptoms and the most severe hypocotyl rot symptoms occurred at different durations of waterlogging of bean plants in this study. This effect has been demonstrated for

other legumes. For example, You et al. (53) noted that resistance to root disease compared with seedling damping-off caused by *P. irregulare* in subterranean clover were different. In that study, damping-off was largely a result of hypocotyl damage whereas root damage was more prevalent on emerging or surviving plants. Similarly, Schmitthenner (42) also reported that resistance was different against hypocotyl rot compared with root disease in soybean caused by *Phytophthora megasperma* var. *sojae*. Together, these studies indicate an opportunity to identify and combine potential resistances to hypocotyl and root disease in common bean caused by *Pythium irregulare*. Combined resistance to hypocotyl and root infection would be an effective means to minimize the impacts of hypocotyl and root disease caused by *P. irregulare* and possibly other *Pythium* spp.

The three bean varieties evaluated in this study responded differently in terms of disease development after waterlogging, with the least hypocotyl and root disease observed on Gourmet Delight and the most severe on Brown Beauty and Pioneer. Despite obvious susceptibility to hypocotyl and root disease, Pioneer overall had the best emergence and shoot dry weight among the three varieties tested, indicating that this variety may have a degree of tolerance not only to each of the waterlogging and hypocotyl or root disease stresses but also to the combined stresses of waterlogging and infection by *P. irregulare*. The nature of this potential tolerance is not known. However, in previous studies in which seed weights across 18 plant species from 15 families ranged from 0.043 to 22.200 mg, and were planted under different soil water conditions (28), seedlings that developed from larger seed had greater emergence and better survival rates, resulting in improved overall seedling establishment compared with smaller seed (28,49). In other studies, a larger seed size resulted in larger seedlings and provided more energy for seedlings to resist waterlogging (46) or disease caused by *P. irregulare* (M. J. Barbetti, *data not published*). In this study, Pioneer had the largest seed size, with a mean weight of 0.41 g/seed, compared with Brown Beauty at 0.29 g/seed and Gourmet Delight at 0.24 g/seed. Pioneer seedlings demonstrated the ability to tolerate both waterlogging stress and root and hypocotyl disease, which may be related to greater capacity of the larger seed size to tolerate biotic and abiotic stress during germination and early seedling growth. Regardless, although the resistance observed in Gourmet Delight seedlings to hypocotyl and root infections by *P. irregulare* could be exploited to develop bean varieties that develop less hypocotyl and root disease in waterlogged conditions, the tolerance in Pioneer also could be exploited to develop varieties that incur less growth impediment as a consequence of hypocotyl or root disease or waterlogging. Future studies should include assessment of bean growth and yields under field conditions that include these biotic and abiotic stresses to verify the responses observed in this study in a growth room, because the simultaneous analysis of yield components in common bean is the most productive avenue to superior selection efficiency for breeders (4).

Biotic-abiotic stress combinations can prevent establishment and high productivity in common bean crops across much of the world's major bean-producing regions, contributing to food insecurity, particularly in sub-Saharan Africa, where bean is a staple food (11,36). The most significant losses often result from interactions between abiotic stresses, particularly excessive or deficient water supply, and biotic stresses such as *Pythium* spp.; therefore, combined resistance to abiotic and biotic stresses should be emphasized. Pathogen diversity and specific pathogen threats are changing under climate change conditions, and may increase (6), making the development of new varieties with both resistance and tolerance to such stresses essential for effective management of soil-borne diseases caused by *Pythium* spp. on common bean. For example, projected increases in rainfall in eastern Africa will not only challenge common bean production directly as a result of increased waterlogging events and durations but also promote pathogens such as *Pythium* spp., posing a greater threat to intensified bean production in regions such as Rwanda and Kenya (11). Fortunately, there has been a renewed emphasis on breeding for increased re-

sistance to multiple environmental (biotic or abiotic) and climate-change-related stresses, and efforts to enhance access to such improved dry bean varieties (16).

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