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REACTIONS AND YIELD PERFORMANCE OF THREE POTATO CULTIVARS NATURALLY INFECTED WITH BACTERIAL BLACKLEG DISEASE UNDER IRRIGATION PRACTICE

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Abstract
Potato (*Solanum tuberosum*) has become an attractive winter crop in many arid irrigated areas where they are grown on relatively large commercial farms. Two field trials were conducted to assess Irish potato cultivars for their tolerance/reactions to natural infection of bacterial blackleg disease under irrigation practice. The experiment was laid out in a randomized complete block design with three replications. The incidence of blackleg disease in both first and second trials ranged from 1.70 to 100% with the highest (100%) recorded in Mirabel, which was highly significant ($p \leq 0.05$), 1.70% - 5.00% disease incidence recorded for Yellow and 15.00% and 16.70% in Christian Lady in both first trial and second trials respectively. Yellow cultivar had 89.60% and 94.50% emergence and these were significantly higher than 8.30% and 21.10% observed in Mirabel in both trials. The highest yield (2.06t/ha) was observed in Yellow cultivar followed by 1.86t/ha recorded for Christian Lady in second trial. Yellow cultivar was tolerant to bacterial blackleg, followed by Christian Lady, while Mirabel was susceptible. Yellow and Christian Lady cultivars proved to be promising cultivars in drought condition or low rainfall areas. Notwithstanding, Yellow cultivar was the most preferable for disease tolerant in drought conditions.

Keywords: Drought, incidence, potato, severity, tolerance

Introduction

Irish potato (*Solanum tuberosum* L.) is classified within the family Solanaceae and is by far the most efficient tuber crop in terms of tuber yield and days to maturity. It matures in about 80 - 90 days as compared to 9 - 12 months for yam and cassava (Muinat and Damilola, 2017). The crop is produced almost throughout the year because of the short maturity period, making it the highest yielding tuber crop (Okonkwo *et al*., 1995a). It is a starchy tuber which has become an attractive winter crop in many arid irrigated areas where they are grown on relatively large commercial farms. In Nigeria for instance, the crop is being cultivated in commercial quantities in two States namely; Plateau (Bokkos, Mangu, Barkin-Ladi, Jos South, Bassa, Riyom, Jos-North and Pankshin LGAs) and Taraba (Sarduna L.G.A. and Mambilla Plateau) (Okonkwo *et al.,* 1995b; Dimlong, 2012). It can be grown in far northern States of Nigeria during the cold harmattan season under irrigation. In 2012, the world production of potato was close to 365 million tons and the area of cultivation was approximately 19.2 million hectares (FAO, 2012). Haile and Boke (2011) reported that18.60 million hectares were dedicated for Irish potato cultivation worldwide in 2010 and the average world farm yield was 17.40t/ha. In 2007, African countries had 1,541,498 ha potato production areas and the average yield was 10.80t/ha, while Nigeria had a total land area of 270,000ha for the same purpose with average yield of 3.10t/ha (FAOSTAT, 2008). Potato is grown for food, animal feed, industrial uses and for seed tuber production (Fikre, 2018). The main use is still as direct food, but an increasing proportion is processed into snack. Potato is important for subsistence farmers but may also be a cash crop (Getachew *et al*., 2012).

Among the diseases attacking Irish potato include, Blackleg (*Pectobacterium* spp.). The major cause of blackleg is *P. atrosepticum*; a tuber/seed-borne pathogen, under cooler summer conditions (De Boer and Rubio, 2004). Blackleg occurs in fields and infects at all stages of plant development. *It* has a narrow host range and predominantly infects potato, whereas related species such as *Pectobacterium carotovorum* and *Dickeya* spp. have a broad host range. Some species such as *P. atrosepticum* survives poorly in soil, water, and plant debris; others such as the *Dickeya* spp. survive much better. The main mode of blackleg infection is through the secretion of pectolytic and other plant cell wall degrading enzymes via the type II secretion system. When grown on a medium containing sodium polypectate, the pectobacteria and dickeya develop pits

or craters in the medium, due to the excretion of pectolytic enzymes that dissolve the pectate. It is due to these pectolytic enzymes and genetic composition that the genus, *Pectobacterium*, was designated. Since *P. atrosepticum* was initially described as the sole cause of blackleg, it is the most extensively studied blackleg-causing bacteria. Losses from infection include, yield reduction. Among the important component of Integrated Disease Management (IDM) is the use of disease resistant/tolerant cultivars and this can have impact on the production, where soils are highly infested (French *et al*., 1998). Some cultivars that seem to have been found to have some degree of tolerance to bacterial pathogen still transmit latent infection to their progeny tubers (French, 1996). Breeding programmes to determine tolerant Irish potato cultivars to blackleg disease are yet to be identified in Nigeria (Ateka *et al*., 2001). Some cultivars have been reported to exhibit variable reactions in different seasons (French, 1994). The use of resistant varieties is the oldest, cheapest and overall best means of controlling plant diseases. This study was therefore, to examine the incidence, severity and yield performance of three Irish potato cultivars under irrigation practice in Kashere, Northeastern Nigeria.

Materials and Methods

Description of the study area

The experiment was conducted at the Teaching and Research farm, Federal University, Kashere, Gombe State. Kashere is located at an elevation of 431 meters above sea level and its coordinates are 9°46 ′0″ N and 10°57′0″ E. Gombe State generally, is characterised by strong rainfall seasonality with distinct wet and dry season (Abashiya *et al.,* 2017). The rainfall is concentrated between the months of May and October with a single maximum in August (Amos *et al*., 2015; Yahaya, 2015). The average annual rainfall total is about 863.20mm (Abashiya *et al*., 2017). The arrival of the rainy season starts around May however, heavy rainfall may appear in June or July (Yahaya and Adamu, 2016) and by October, the amount of rainfall begins to decline (Yahaya, 2015). The mean annual temperature is about 32°C. Weather tends to be very hot between November and March, with average high temperature that can reach 32°C or more (Yahaya and Adamu, 2016). Harmmatan wind from around February makes the temperature cooler, while relative humidity can be up to 90% in August but drops during the hammattan period (Balzerek *et al*., 2003).

Experimental Design

The two trials experiment consist of three potato cultivars (Christian Lady, Yellow and Mirabel) were obtained from National Root Crops Research Institute, Vom, Plateau State, Nigeria. The experiment was laid out in a randomized complete block design with three replications, and repeated once. Land was manually prepared and beds of 4m x 4m in dimension constructed with 1m pathway. Plant spacing of 70 by 30cm was used. Planting was done in January, 2018 and repeated in March the same year. Water was applied as required.

Isolation, pathogenicity and pectolytic activity of Pectobacterium atrosepticum

(i)Isolation

Samples of infected potato stem with blackleg symptoms (wilting, internal and external darkening on stems) were collected from the experimental field. Infected stems were taken to laboratory, washed under running tap water, sterilized with 5% sodium hypochlorite, washed in three changes of sterile distilled water and macerated in 0.01M magnesium sulphate ($MgSO_4$). Isolation was done on the selective medium, Crystal Violet Pectate (CVP) (Hyman *et al.,* 2001) and plates incubated at temperature of 28±2ºC for 48hrs. Pure cultures were tested positive for pectolytic cavity formation and transferred to nutrient agar (NA) slant, on which they were maintained at -4ºC for further use.

(ii)Pathogenicity test on potato tuber

Healthy potato tubers were washed under running tap water, surface sterilized in 70% ethanol and washed in three changes of sterile distilled water. Three millimeters (3mm) diameter size sterile cork borer was used to cut out 5mm deep cylindrical tissues on the tubers. One milliliter (1ml) of bacterial isolate suspension (10^8cfu/ml) was inoculated into each hole. The control tubers were inoculated with sterile distilled water. Tubers were left for two hours to allow suspension sink into tuber tissue before replacing the cut out cylindrical tissue. The inoculation points were sealed up with petroleum jelly. Incubation was for two weeks at room temperature.

(iii)Pectolytic test on potato tuber slices

Bacterial isolates were tested for pectolytic activity on potato tubers (*Solanum tuberosum* cv Mirabel). The tubers were washed under running tap water, air dried and then dipped in 70% ethanol, flamed and cut into 10mm thick slices. The slices were placed on wet sterile filter papers in Petri dishes. At the center of potato slice, a groove was created and two loopful (10^8cfu/ml) of bacterial isolate dropped in each nick of potato slices. Development of rot on the slices was examined 24–48h after incubation at 28±2ºC. Control tubers were treated in the same manner but with sterile distilled.

Data collection and analysis

Disease severity was assessed on a scale of 0-3 as reported by Wright *et al*. (2005) where, 0 = no disease symptoms on plant, 1 = ≤ 50% of the plant has disease symptoms, 2 = > 50 % of the plant has disease symptoms, and 3 = plant totally dead. Data were collected on disease incidence, severity, plant height (cm), number of leaves/plant, number of branches/plant and yield (t/ha). Data were subjected to analysis of variance using GenStat Discovery Edition 4. Means were separated via LSD at $p \leq 0.05$.

Results and Discussion

In Table 1, sand accounted for 63.60% of the texture; silt and clay were 22.40% and 14.00%, respectively. This implies that the soil was moderately porous and sandy loam in nature. During the experiment, there was very low rainfall. No rain in January all through to March, while insignificant rainfalls of 22.47, 21.63 and

19.68mm were received in April, May and June, respectively (Table 2). High relative humidity was witnessed in June (81.21%) (Table 2). Mean soil temperature at 10cm depth ranged from 30.00°C to 38.17°C and at 20cm from 23.79°C to 31.37°C (Table 2). Pure culture inoculated into healthy tubers produced rot symptom similar to that observed under natural conditions and the organism isolated from it developed deep pits in Crystal Violate Pectate (CVP) medium, which is the feature of *Pectobacterium atrosepticum.*

Table 3 shows the results of the assessment of variability in growth parameters of the three genotypes of potato used in the study. There were significant differences ($p \leq 0.05$) in all the parameters measured. Number of leaves/plant ranged from 22.00 to 79.60 in both trials with the highest number of leaves/plant (79.60) recorded in Yellow cultivar in second trial, followed by Christian Lady with 79.10 number of leaves/plant. These were significantly higher ($p \leq 0.05$) than 22.00 and 26.20 observed in Mirabel cultivar in both first and second trials respectively. Number of branches/plant ranged from 5.00 to 23.33. In second trial, the highest number of branches/plant (23.33) was recorded in Yellow cultivar followed by Christian Lady with 23.00, which was significantly higher ($p \leq 0.05$) than 5.00 and 8.00 observed in Mirabel. Mirabel had the lowest plant height (10.67 and 13.67cm), number of leaves/plant (22.00 and 26.20) and number of branches/plant (5.00 and 8.00) throughout the period of experiment in both first and second trials respectively.

Cultivars showed highly significant differences ($p \leq 0.05$) in disease incidence, severity, germination percentage and yield (Table 4). Blackleg incidence ranged from 1.70 to 100% with the highest disease incidence (100%) recorded in Mirabel during the first trial and was highly significant ($p \leq 0.05$) from disease incidence observed in Yellow (5.00% and 1.70%) and Christian Lady (15.00% and 16.70%) in both first and second trials respectively. Likewise, Mirabel had highest disease severity of 3.00 in both trials and was significantly ($p \leq 0.05$) higher than severity recorded for Christian Lady (1.00) and Yellow (0.67 and 0.33) cultivars in both first and second trials respectively.

In the first and second trials, Yellow cultivar was outstanding in percent emergence of 89.60% and 94.50% respectively, though not significantly different ($p \geq 0.05$) from percent emergence (63.70% and 73.60%) recorded for Christian Lady, but were both significantly higher ($p \leq 0.05$) than 8.30% and 21.10% observed in Mirabel respectively (Table 4). Yield among cultivars ranged from 0.04 to 2.06t/ha. The highest significant yield (2.06t/ha) was observed in Yellow cultivar in the second trial followed by 1.86t/ha recorded for Christian Lady. Figure 1 shows the progression of blackleg incidence on potato cultivars in first (Fig. 1A) and second (Fig. 1B) field trials at 4, 8 and 12 weeks after emergence (WAE). In the first trial (Fig. 1A), incidence ranged from 5.00% to 100%. There was total incidence (100%) on Mirabel at 12WAE, while Yellow had the least incidence (5.00%) and significantly different ($p \leq 0.05$) from one another. The same trend was observed during the second trial (Fig. 1B). In Fig. 2A, Mirabel had highest disease severity of 2.00 and 3.00 in the first field trial at 8 and 12WAE, which were significantly different ($p \leq 0.05$) from 1.00 and 0.67 observed in Christian Lady and Yellow cultivars, respectively. Also, all cultivars had the same trend of severity recorded in the second field trial (Fig. 2B).

Out of the three Irish potato cultivars used in this study, two were found to be tolerant to blackleg disease and thus, attested promising for Irish potato farmers in arid areas where irrigation is being practiced. Felix *et al.* (2010) reported the differences in reactions of Irish potato to bacterial wilt disease. Since *P. atrosepticum* is a tuber-borne pathogen, differences in resistant status of the cultivars used might be due to the inherent genetic variability. This is supported by French (1994), who observed that potato cultivars could exhibit different reactions to pathogens even within an environment. Mirabel showed highest disease incidence which was the indicator of serious disease severity compared with Christian lady and Yellow, which could be attributed to soil higher moisture. This is because, as asserted by Van Elsas, *et al*. (2005), soil moisture was a very crucial factor in disease occurrence. Temperature was near optimum which could have led to high disease severity experienced in Mirabel cultivar. Latent infection that resulted in Christian lady and Yellow cultivars signified their tolerance to blackleg disease development. Percentage emergence was significantly different. This had been explained by Ochuodho and Modi (2006) in their logistic regression models on seed germination process and temporal aspects of disease epidemiology (Ameson, 2006). Results obtained in this study revealed that Mirabel cultivar was most susceptible to blackleg disease and this was consistent in both trials. High tuber infection and percent disease incidence and severity had significant influence on Mirabel, which translated to low yield.

Conclusion

The presence of significant differences among cultivars indicated their genetic variability to disease resistance. Though, all varieties yielded below national average yield, still, Christian Lady and Yellow cultivars indicated their promising potentials in drought condition or low rainfall areas and yielding more under rain-fed condition where they could be able to explore their genetic potential maximally. Notwithstanding, Yellow cultivar was the most tolerant. However, research between years and agro-ecological zones on the cultivars used in this study are recommended.

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Table 1: Physico-chemical characteristics of the soil at the experimental site

Soil characteristics	Value
Percent clay	14.00
Percent silt	22.40
Percent sand	63.60
Soil Texture	Sandy loam
pH	5.80
Organic carbon (%)	0.25
Total nitrogen (%)	0.02
Available phosphorus (mg kg^{-1})	6.63
CEC (Cmol kg^{-1} soil)	16.17
Exchangeable bases (Cmolkg^{-1}soil)	
Ca^{2+}	12.00
Mg^{2+}	3.10
K^{+}	0.23
Na^{+}	0.04

Source: (Audu, 2017)

Table 2: Weather data of the experimental site in the year 2018

Month	Rainfall (mm)	Soil temperature (ºC)		Relative humidity (%)
		Mean (10 cm depth)	Mean (20 cm depth)	
January	0	30.00	23.79	18.04
February	0	30.40	23.83	19.67
March	0	38.17	31.37	31.50
April	22.47	37.72	30.87	58.33
May	21.63	34.94	28.35	60.50
June	19.68	31.41	25.68	81.21

Table 3: Assessment of differences in plant height (cm), number of leaves/plant and number of branches/plant of potato cultivars

Cultivar	Plant height(cm)		Number of leaves		Number of branches	
	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
Christian Lady	26.00	29.04	75.50	79.10	20.00	23.00
Yellow	27.53	30.69	76.60	79.60	20.33	23.33
Mirabel	10.67	13.67	22.00	26.20	5.00	8.00
$LSD_{(0.05)}$	4.28	4.33	30.76	31.37	1.96	5.07

Table 4: Bacterial blackleg incidence and severity, emergence (%) and tuber yield (t/ha) as influenced by Irish potato cultivars

Cultivar	Incidence (%)		Severity		Emergence (%)		Yield (t/ha)	
	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
Christian Lady	15.00	16.70	1.00	1.00	63.70	73.60	1.71	1.86
Yellow	5.00	1.70	0.67	0.33	89.60	94.50	1.82	2.06
Mirabel	100.00	98.30	3.00	3.00	8.30	21.10	0.04	0.21
$LSD_{(0.05)}$	15.35	12.81	0.76	0.76	33.50	23.30	0.47	0.48

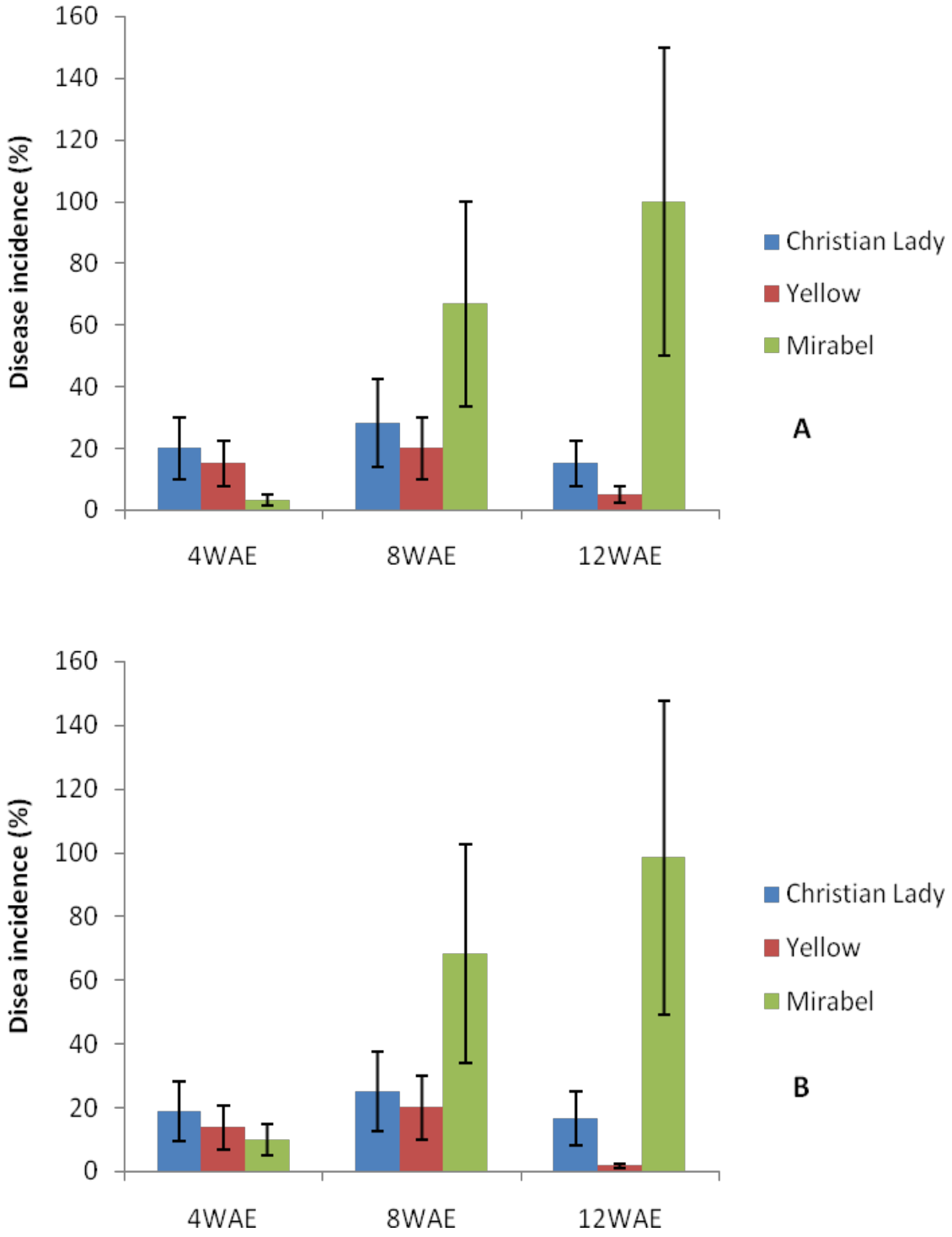

Figure 1: Incidence of blackleg disease on Irish potato cultivars in the first (A) and second (B) field trials at 4, 8 and 12 weeks after emergence (WAE)

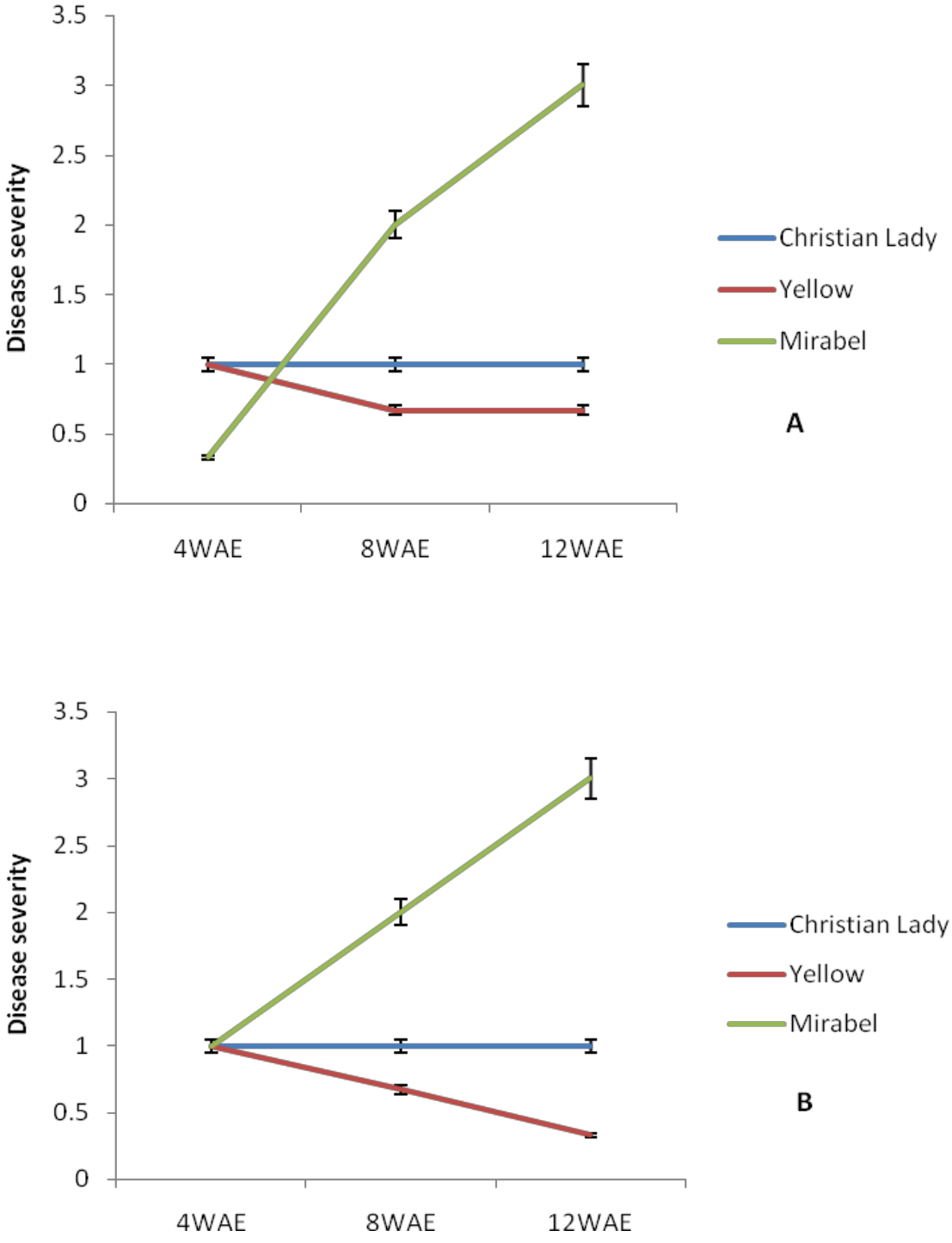

Figure 2: Severity of blackleg disease on Irish potato cultivars in the first (A) and second (B) field trials at 4, 8 and 12 weeks after emergence (WAE)