Pathogenicity and Aggressiveness of *Fusarium oxysporum* Schl. **in Bottle Gourd and Bitter Gourd**

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Abstract

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Fusarium oxysporum is responsible for a large range of diseases on economically important crops such as bitter gourd and bottle gourd. Pathogenicity and aggressiveness of *F. oxysporum* in bitter gourd and bottle gourd isolated from two breeding stations of East-West Company in the Philippines namely San Ildefonso, Bulacan and Lipa, Batangas were tested. Eleven *F. oxysporum* isolates from bitter gourd and 12 isolates from bottle gourd were inoculated on 7-day and 1-month-old bitter gourd and bottle gourd plants in the greenhouse. All *F. oxysporum* isolates from bitter gourd were pathogenic on 7-day-old and 1-month-old bitter gourd and nine out of 12 isolates from bottle gourd were pathogenic on bottle gourd. Three isolates from the infested soil were non-pathogenic on bottle gourd. There was a significant difference in aggressiveness of the isolates on their natural hosts ($P \leq 0.05$). There also was a significant difference in the aggressiveness of isolates pathogenic on bitter gourd from Batangas and Bulacan ($P \leq 0.05$) but isolates from Batangas and Bulacan had similar aggressiveness as bottle gourd ($P \geq 0.05$). Aggressiveness of *F. oxysporum* on 7-day-old bitter gourd and bottle gourd was significantly different compared to those on 1-month-old plants, demonstrating an effect of the host age onaggressiveness. Correlations between aggressiveness of *F. oxysporum* isolates on 7-day-old and 1-month-old bitter gourd and bottle gourd were moderate ($r = 0.63, 0.78$). Out of 12 isolates from bottle gourd, only one isolate was pathogenic on 7-day-old bitter gourd. Four of the isolates from bitter gourd were pathogenic on 7-day-old bottle gourd but not on 1-month-old bottle gourd. No cross infection was observed on mature plants.

Keywords: bitter gourd; bottle gourd; *Fusarium oxysporum*; *Lagenaria ciceraria*; *Momordica charantia* Linn.; pathogenicity; aggressiveness

Bottle gourd (*Lagenaria ciceraria*) and bitter gourd (*Momordica charantia* Linn.) of the family Cucurbitaceae are common vegetables grown throughout the Philippines for food and medicinal properties (Bureau of Plant Industry 2006). Members of this family are susceptible to Fusarium wilt caused by different formae speciales of *Fusarium oxysporum. F. oxysporum* was the first of nine species described in the section Elegans (Booth 1971). The majority of the wilt fusaria are host specific strains capable of attacking only a single plant species and were differentiated from each

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other by formae speciales (f.sp.) (Summerell *et al.* 2003). The formae speciales are based on their strict host specificity (Snyder & Hansen 1940).

Formae speciales of *F. oxysporum* are identified by their pathogenicity and virulence on a set of host differentials appropriate for the forma specialis in question. Pathogenicity is defined as the ability of an organism to cause disease (Horsfall & Dimond 1960) while aggressiveness refers to the disease severity of an isolate on different hosts or on hosts of different age.

Fusarium wilt of bottle gourd is caused by *Fusarium oxysporum* f.sp. *lagenariae* (Matuo & Yamamoto 1957) while fusarium wilt of bitter gourd is caused by *F. oxysporum* f.sp. *momordicae* nov. f. (Sun & Huang 1983). While host specificity of formae speciales is typical, there are a few cases of cross-species infectivity. The pathogenicity of *F. oxysporum* from Cucurbitaceae is non-specific particularly when inoculated as seedling stage (Gerlagh & Blok 1988). Kim *et al.* (1993) showed cross infectivity of *F. oxysporum* f.sp. *cucuminerum* isolated from wilted cucumber to muskmelon and watermelon. *F. oxysporum* f.sp. *niveum* isolates from watermelon (*Citrullus lanatus*) were also found to infect *Cucurbita pepo* cultivars (Martyn & McLaughlin 1983). Bouhot (1981) also reported that one forma specialis could be induced to mutate to another forma specialis and become pathogenic to another host. Cross infectivity of formae speciales is generally only in seedlings as host specificity is the rule in mature plants (Owen 1956; Armstrong & Armstrong 1978; Gerlagh & Blok 1988).

Aside from the initials reports (Matuo & Yamamoto 1957; Sun & Huang 1983) little information on the pathogenicity and aggressiveness of *F. oxysporum* f.sp. *lagenariae* and *F. oxysporum* f.sp. *momordicae* is available. This knowledge would be important in evaluating risks associated with management strategies, selecting strains used in resistance screening, and developing strategies for deployment of resistance. This is particularly important as bottle gourd is often used as a rootstock for grafting watermelon and melon to control their respective fusarium wilt diseases.

This objectives of this study were (1) to determine the pathogenicity of *F. oxysporum* isolates in bottle gourd and bitter gourd collected from two breeding stations of the East-West Company in the Philippines; namely San Ildefonso, Bulacan and Lipa, Batangas; (2) to compare the aggressiveness of *F. oxysporum* isolates in young and mature bottle gourd and bitter gourd plants; and (3) to determine the cross infectivity of *F. oxysporum* isolates in bottle gourd and bitter gourd.

Table 1. *Fusarium oxysporum* isolates, their hosts and origin

Isolate code	Host/source	Origin breeding station
$A_2$16		San Ildefonso, Bulacan
$A_2$18		San Ildefonso, Bulacan
$A_1$2		Lipa, Batangas
$A_1$7		Lipa, Batangas
$A_1$10		Lipa, Batangas
$A_1$11		Lipa, Batangas
$A_1$12		Lipa, Batangas
$A_1$17		Lipa, Batangas
$A_1$20	bitter gourd/stem	Lipa, Batangas
Fo-Am1		Lipa, Batangas
Fo-Am14		Lipa, Batangas
$U_2$8		San Ildefonso, Bulacan
$U_2$16		San Ildefonso, Bulacan
$U_1$4		Lipa, Batangas
$U_1$5		Lipa, Batangas
$U_1$12		Lipa, Batangas
$U_1$14		Lipa, Batangas
$U_6 10^2$		Lipa, Batangas
$U_9 10^6$	bottle gourd/soil	Lipa, Batangas
$U_{10} 10^6$		Lipa, Batangas
Fo-Up10		San Ildefonso, Bulacan
Fo-Up15	bottle gourd/stem	Lipa, Batangas
Fo-Up25		Lipa, Batangas

MATERIALS AND METHODS

Culture and maintenance of *Fusarium oxysporum.* Single spore isolates of *F. oxysporum* isolated from bitter gourd and bottle gourd (Table 1) at the two breeding stations were cultured on Spezieller Nährstoffarmer Agar (SNA) (Nirenberg 1976).

For long term storage, the isolates were cultured on pieces of sterile filter paper in potato dextrose agar (PDA) at room temperature, under fluorescent light for at least one week. Afterwards, the colonised filter paper was transferred into sterile Petri dishes and dried at room temperature, and then stored in sterile Eppendorf tubes at 4°C (McCallum *et al.* 2001).

Preparation of plant materials. Seeds of bottle gourd var. Tambuli and bitter gourd var. Jadestar (East-West Seed Company, San Ildefonso, Bulacan) were sown in plastic seedling trays containing sterilised potting soil. After 7 days, the seedlings were lifted out of the trays and their roots were dipped into the prepared inoculum suspension for 15 min, transplanted into plastic pots and allowed to grow for 14 days. For one-month-old plants, seeds of bottle gourd and bitter gourd were sown in plastic seedling trays with sterilised soil. After seven days, the seedlings were transplanted into plastic pots and were grown in the greenhouse for another 21 days. One-month-old plants were predisposed to infection by wounding the roots and base of the plants using a flame-sterilised scalpel at the time of inoculation.

Inoculum production and inoculation. *F. oxysporum* isolates collected from Bulacan and Batangas were cultivated on filter discs on oatmeal agar for 14 days under continuous light. Conidial suspensions of each isolate were prepared by removing the filter discs from the plates and placing them in plastic sachets. Sterile distilled water was added into the sachet and the mycelial growth on the filter paper was scraped off using a glass rod. The suspension was filtered through two layers of sterile cheesecloth and the conidia were adjusted to 10^6 conidia per ml using a hemacytometer. The roots of the 7-day-old plants were dipped into 50 ml of 1×10^6 conidia for 15 min and transplanted to pots. The base of one month old plants were inoculated by pouring 50 ml of the conidial suspension into the pots. Parts of the wounded roots were exposed to the conidial suspension by removing some soil and covering the roots again after inoculation. Control plants were treated with 50 ml sterile distilled water. All plants were grown in the greenhouse for 21 days.

Disease assessment. A rating scale of 1 to 4, where 0 represented plants that had no symptoms, 1 for plants that showed vein clearing alone, 2 for plants that were partially wilted, and 3 for plants that were completely wilted was used to measure the aggressivenss of each isolate. The same rating scale was used for evaluating the 1-month and 7-day-old bitter gourd and bottle gourd plants. Ratings were done at 7, 14 and 21 days after inoculation (DAI).

Analysis of data. All treatments were a randomised complete block design (RCBD) with ten replications per isolate. Tests for significant differences among isolates and between the different ages of plants was analysed using the SAS software version 6.12 (SAS 1996).

RESULTS AND DISCUSSION

Pathogenicity and aggressiveness of *F. oxysporum* isolates on bitter gourd and bottle gourd

Eleven *F. oxysporum* isolates from bitter gourd and 12 isolates from bottle gourd were used in the experiment (Table 1). All bitter gourd isolates produced typical symptoms of vein clearing and wilting. At 21 DAI, three isolates from Batangas and one isolate from Bulacan caused wilting of both 7-day-old and 1-month old bitter gourd. The symptoms were the same as those described by MacHardy and Beckman (1981) and Sun and Huang (1983) in bitter gourd.

Table 2. Means of disease severity of bitter gourd caused by *F. oxysporum* isolates 21 days after inoculation (DAI)

Isolate code	Disease severity
$A_1$7	2.9^{a}
$A_1$2	2.25^{b}
$A_2$16	2.15^{bc}
$A_1$17	1.75^{bcd}
$A_1$12	1.7^{cd}
Fo-Am14	1.65^{cd}
$A_2$18	1.5^{d}
$A_1$10	1.45^{d}
Fo-Am1	0.85^{e}
$A_1$11	0.45^{ef}
$A_1$20	0.25^{f}
Control	0^{f}

Means with the same letter are not significantly different using Least Significance Difference Test at $P \leq 0.05$

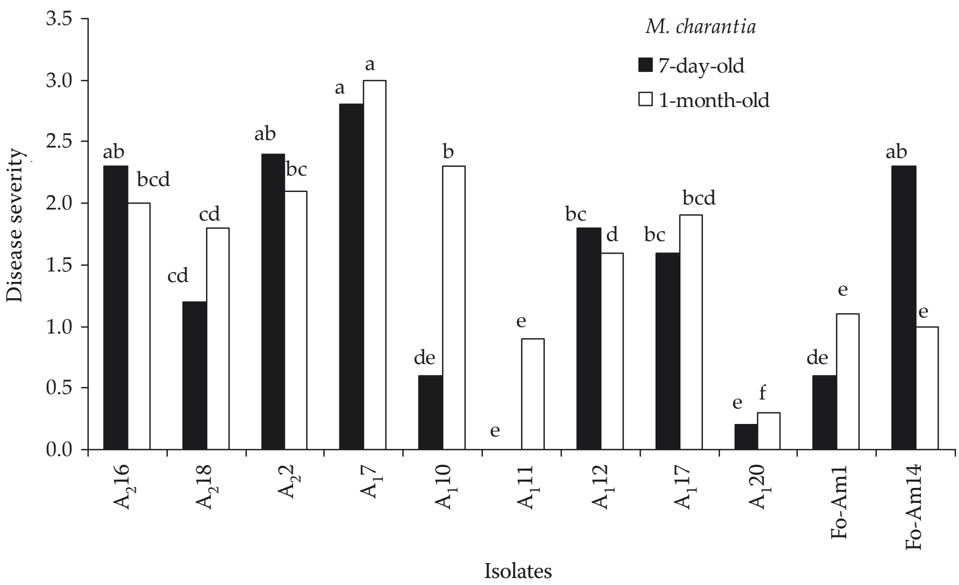

Figure 1. Disease severity caused by *F. oxysporum* isolates on bitter gourd 21 days after inoculation (DAI) (Isolates with the same letters within the same plant age are not significantly different with $P \le 0.05$ using LSD)

The aggressiveness of *F. oxysporum* isolates from bitter gourd differed significantly ($P \le 0.05$). Isolate $A_1$7 was the most aggressive whereas $A_1$11 and $A_1$20 were considered non-pathogenic to both 7-day and 1-month-old bitter gourd (Table 2). Isolates $A_1$11 was least aggressive among the isolates inoculated to 7-day-old bitter gourd while $A_1$7, was the most aggressive (Figure 1). Isolate $A_1$20 was the least aggressive among the isolates inoculated to 1-month old and $A_1$7 the most aggressive. Isolate $A_1$7 was aggressive in both host age (Figure 1).

Nine of the 12 *F. oxysporum* isolates from bottle gourd also caused vein clearing and wilting. Two isolates from Batangas and one isolate from Bulacan caused wilting. Vein clearing appeared as early as 7 DAI while wilting began at 14 DAI. The three isolates collected from the soil were non-pathogenic even at 21 DAI.

Disease severity of the isolates from bottle gourd differed significantly ($P \le 0.05$) in both in 7-day and 1-month-old bottle gourd plants. The isolates $U_1$12, Fo-Up10, and Fo-Up15 were the most aggressive whereas $U_2$8 was the least aggressive (Table 3). Isolates $U_2$16, $U_6 10^2$, $U_9 10^6$, and $U_{10} 10^6$ were non-pathogenic. Isolate $U_2$8 also was non-pathogenic to 1-month-old bottle gourd but pathogenic to 7-day-old bottle gourd. (Figure 2). In general, the pathogenic isolates were more aggressive in 7-day-old plants than 1-month-old plants.

Several studies have proposed different mechanisms associated with the virulence of *F. oxysporum*. Enzymes that act upon the pectic and cellulosic components of plant cell walls and which cause

Table 3. Means of disease severity of bottle gourd caused by *F. oxysporum* isolates 21 days after inoculation (DAI)

Isolate code	Disease severity
$U_1$12	2.5^{a}
Fo-Up15	2.3^{ab}
Fo-Up10	2.25^{ab}
$U_1$5	2.1^{bc}
Fo-Up25	2.05^{bc}
$U_1$14	1.95^{bc}
$U_1$4	1.75^{c}
$U_2$8	1.25^{d}
$U_2$16	0.05^{e}
$U_6 10^2$	0^{e}
$U_9 10^6$	0^{e}
$U_{10} 10^6$	0^{e}
Control	0^{e}

Means with the same letter are not significantly different using Least Significance Difference Test at $P \le 0.05$

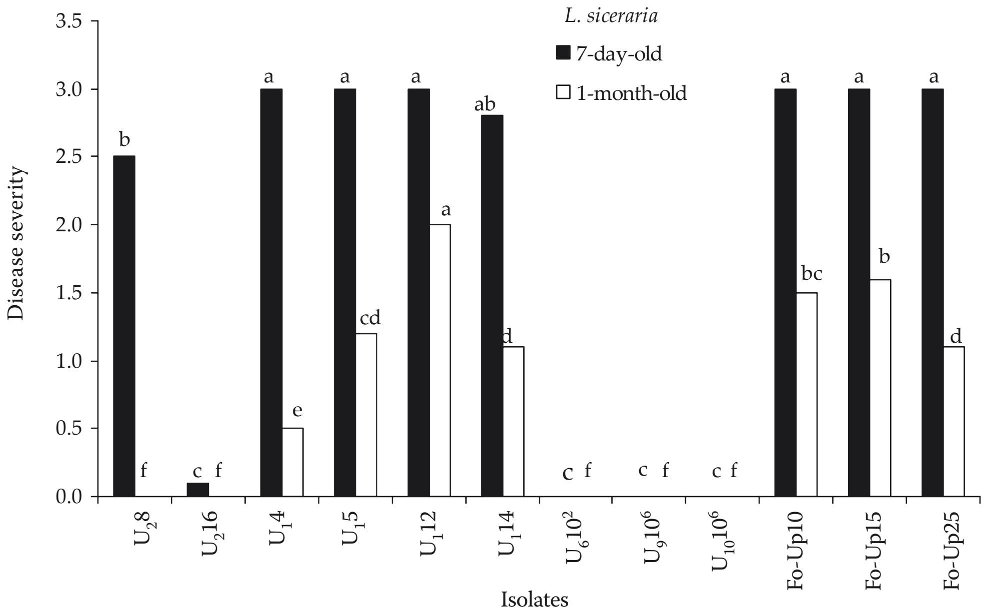

Figure 2. Disease severity caused by *F. oxysporum* isolates on bottle gourd 21 days after inoculation (DAI) (Isolates with the same letters within the same plant age are not significantly different with $P \leq 0.05$ using LSD)

the loss of the cell integrity have been suggested as causing the vascular browning and wilting (Winstead & Walker 1954; Gothoskar *et al.* 1955; Heitefuss *et al.* 1960; Deese & Stahmann 1962). Toxins also have been implicated in a primary role in symptom induction (MacHardy & Beckman 1981). Changes in uninvaded leaves and stems of plants, such as an increase in respiration, alterations in phenol metabolism, activation of polyphenoloxidases, increase in growth regulator activity, and vascular browning are suggested to be due to the actions of toxins (Foster 1946; Davis 1954; Collins & Scheffer 1958).

Effect of plant age on pathogenicity and aggressiveness of *F. oxysporum* isolates

Armstrong and Armstrong (1978) proposed that the stage or age of the plant affects the aggressiveness of *F. oxysporum*. In our study, as early as 7 DAI, typical symptoms induced by *F. oxysporum* were observed on both 7-day and 1-month-old plants. However, the test for the significance showed that the disease severities of the 7-day and 1-month-old bottle gourd caused by *F. oxysporum* were significantly different ($P \leq 0.05$) while those caused by isolates in 7-day and 1-month-old bitter gourd were similar ($P \geq 0.05$). The aggressiveness of the isolate varies depending on the age of the plant. This variation can be attributed to the differences in the mechanisms of host resistance in different stages of the plant (Beckman 1964). Young plants do not exhibit maximum resistance making them more susceptible to infection. Armstrong and Armstrong (1975) used older seedlings in their experiments to avoid damping-off and to assess adult plant resistance.

The correlations between disease severity of 7-day and 1-month old were moderate ($r = 0.63$ for bitter gourd; $r = 0.78$ for bottle gourd) (Figure 3), suggesting that either of the two plant ages can be used for pathogenicity tests.

Cross infectivity of *F. oxysporum* isolates in bitter gourd and bottle gourd

Snyder and Hansen (1940) reported that there is host specificity among the different formae speciales of *F. oxysporum*. However, several studies have showed cross infectivity of some formae speciales to other hosts, especially in the family Cucurbitaceae (Bouhot 1981; Martyn & McLaughlin 1983; McMillan 1986; Gerlagh & Blok 1988; Kim *et al.* 1993).

No symptoms were observed on 1-month-old bitter gourd 21 days after inoculation *with F. oxysporum* isolates from bottle gourd. However, one of the 12 bottle gourd isolates was pathogenic

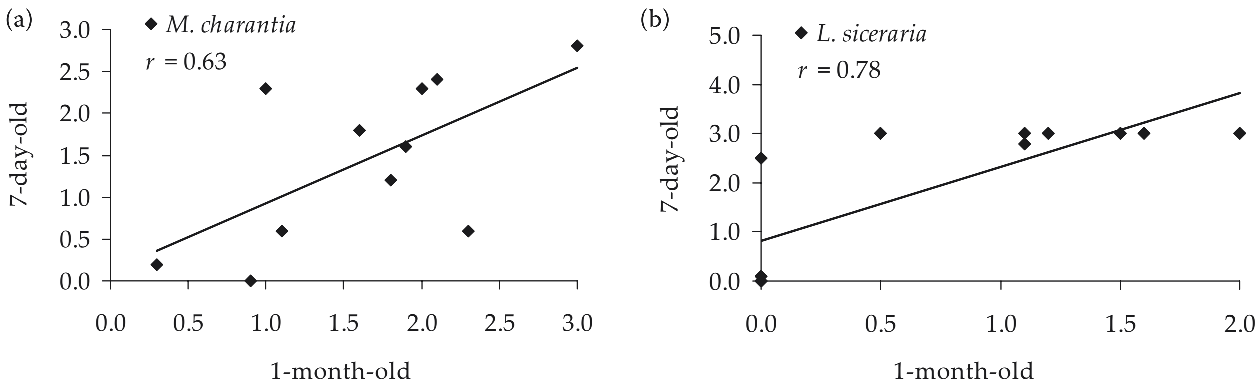

Figure 3. Correlation between disease severity of 7-day and 1-month-old bitter gourd (a) and bottle gourd (b) caused by *F. oxysporum*

on 7-day-old bitter gourd seedlings 14 DAI. This isolate $U_6 10^2$, was isolated from soil and caused vein clearing and wilt in bitter gourd seedlings. This isolate was non-pathogenic on both 7-day and 1-month-old bottle gourd plants. It is possible that bitter gourd is a host of this isolate.

A similar reaction was observed on 1-month-old bottle gourd plants inoculated with *F. oxysporum* isolates from bitter gourd. Plants were still healthy even after 21 DAI. Inoculated 7-day-old seedlings, on the other hand, produced symptoms typical of *F. oxysporum* infection. Four of the 11 bitter gourd isolates caused vein clearing in the young leaves of the 7-day-old seedlings as early as 7 DAI. These four isolates were $A_2 16$, $A_1 2$, $A_1 20$, and Fo-Am1. The fact that *F. oxysporum* isolates from bitter gourd can infect both bitter gourd and bottle gourd suggests that there is a close relationship between the formae speciales infecting these two crops (Kim *et al.* 1993).

A study by Gerlagh and Blok (1988) showed that specificity of formae speciales of *F. oxysporum* causing wilt in the Cucurbitaceae in the seedling stage is limited, thus, cross infectivity occurs. Many of their isolates were pathogenic on seedlings of species or genera other than the one from which they had been isolated. On adult plants, each forma specialis was host- specific. They proposed a new forma specialis 'f.sp. *cucurbitacearum'* which would embraces all formae speciales that attack plants in the family Cucurbitaceae. Moreover, Armstrong and Armstrong (1978) suggested that cucurbitaceous crops are only infected by *F. oxysporum* isolates from plants within the family Cucurbitaceae, and such isolates cannot infect other plants beyond this family.

The isolates used in this experiment came from two breeding stations of the East-West Company in the Philippines, namely San Ildefonso, Bulacan and Lipa, Batangas. The aggressiveness of bitter gourd isolates from the two breeding stations was significantly different but aggressiveness of bottle gourd isolates was similar (Table 4).

The aggresiveness of the isolates that had been isolated within the same breeding station but from different sources (stem or soil) were significantly different. Isolates from the soil ($U_6 10^2$, $U_9 10^6$, and $U_{10} 10^6$) were non-pathogenic on bottle gourd while isolates from the stems of bottle gourd caused

Table 4. Means of disease severity of bitter gourd and bottle gourd caused by *F. oxysporum* isolates from two breeding stations of East West Seed Company 21 days after inoculation (DAI)

Host	Breeding station	No. of isolates	Disease Severity*
Bitter gourd	San Ildefonso, Bulacan	2	1.82[a]
		9	1.32[b]
Bottle gourd	Lipa, Batangas	3	1.18[a]
	San Ildefonso, Bulacan, Lipa, Batangas	9	1.27[a]

*Means with the same letter within the same host are not significantly different using Least Significance Difference Test at $P \leq 0.05$

vein clearing and wilting. It is common to isolate more than one species of *Fusarium* from the same infected plant, while secondary pathogens and saprophytes are more likely to be isolated from intensively cultivated soil (Lim 1972).

Several studies have showed that vegetative compatibility grouping (VCG) and pathogenicity are closely correlated (Bosland & Williams 1987; Larkin *et al.* 1990; Katan *et al.* 1991; Vakalounakis & Fragkiadakis 1999). Cumagun *et al.* (2008) reported four VCGs from *F. o.* f.sp. *momordicae* and five VCGs in *F. o.* f.sp. *lagenariae*.

In our previous study, bitter gourd isolates $A_1$2, $A_1$10, $A_1$11, $A_1$20, $A_2$16 belonged to one VCG whereas $A_2$18 belonged to a separate VCG (Cumagun *et al.* 2008). Among the three bottle gourd isolates analysed for VCG, $U_1$5 belonged to a separate VCG while $U_1$14 and $U_1$4 were of the same VCG (Cumagun *et al.* 2008). There was no observed correlation between VCGs and aggressiveness of the isolates from bitter gourd and bottle gourd analysed for VCG.

No association between VCGs and aggressiveness of bitter gourd and bottle gourd isolates of known VCG (Cumagun *et al.* 2008) suggests that isolates that belong to the same VCG do not necessarily imply that they have similar aggressiveness. However, several studies indicate a positive correlation between the VCG and virulence of *F. oxysporum* isolates. For example, Correll *et al.* (1986) showed that the virulence *F. oxysporum* f.sp. *apii* isolates was found to be correlated with VCG. Vakalounakis *et al.* (2004) and Ahn *et al.* (1998) also found out that the virulence of *F. oxysporum* f.sp. *cucumerinum* in China and Korea was associated with VCG. Likewise, a good correlation between VCG and pathogenicity and race of *F. oxysporum* f.sp. *niveum* was also found by Larkin *et al.* (1990). Information regarding pathogenicity and aggressiveness *of F. oxysporum* from this study will serve as a baseline information on the future prospects of breeding for fusarium wilt resistance in bitter gourd and bottle gourd in East-West Seed Company, Philippines.

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