Interaction between Fusarium oxysporum f. sp. conglutinans and Turnip Mosaic Virus in Brassica campestris var. chinensis Seedlings

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The authors thank H. W. Neufeld for technical assistance and G. A. Mulligan (Plant Research Institute, Ottawa, Canada) for identifying *Brassica campestris* var. *chinensis*. They are also grateful to W. G. Kemp and I. R. Evans (University of Guelph, Ontario, Canada) for supplying turnip mosaic virus isolates I and II, respectively.

Accepted for publication 7 June 1972.

ABSTRACT

The severity of cabbage yellows symptoms induced in *Brassica campestris* var. *chinensis* by *Fusarium oxysporum* f. sp. *conglutinans* was increased when plants were infected simultaneously with turnip mosaic virus. Fungus-infected plants inoculated with the virus were more severely yellowed and weighed less than plants infected with the fungus alone, regardless of the interval between fungus and virus inoculations. Similarly, mosaic-infected plants when inoculated with the fungus were more severely stunted than plants infected with the virus alone. Interaction was greatest at a soil temperature of 28 C.

Phytopathology 62:1424-1428

Additional key word: pak-choi.

Fusarium oxysporum (Schlecht.) emend. Snyd. & Hans. f. sp. *conglutinans* (Wr.) Snyd. & Hans. is a destructive pathogen on crucifers in Ontario. Turnip mosaic virus (TuMV) also infects a wide range of crucifers in Ontario, especially *Brassica campestris* L. var. *chinensis* (L.) Makino (commonly known as pak-choi), a vegetable grown mainly for the Chinese food market.

Preliminary tests indicated that eight isolates of *F. oxysporum* f. sp. *conglutinans* collected from infected crucifers in the field were severely pathogenic on the susceptible cabbage (*B. oleracea* var. *capitata* L.) 'Early Marvel', and moderately so on *B. campestris* var. *chinensis*. Two isolates of TuMV were pathogenic on all cultivars recommended to growers in Ontario (10) except radish (*Raphanus sativus* L.).

Virus infection in plants is known to affect severity of fungus root diseases of many leguminous and cereal crops (2, 3, 6, 8). In some cases, the interaction in the plant is only slight or not apparent (7). Our surveys of Ontario fields since 1967 revealed that both *F. oxysporum* f. sp. *conglutinans* and TuMV may occur in the same field and attack a common vegetable plant. This paper presents the results of tests to determine the interaction occurring between *F. oxysporum* f. sp. *conglutinans* and TuMV in *B. campestris* var. *chinensis* seedlings under controlled environmental conditions.

MATERIALS AND METHODS.—*Seedling*

culture.—Seeds of *B. campestris* var. *chinensis* produced in the greenhouse on plants collected from commercial plantings were sown in a steam-treated mixture of peat moss, sandy loam, and sand (1:3:1) in a steam-treated 7 X 31 X 39 cm wooden box. Forty-eight seeds were sown per box kept in a greenhouse or propagation room for 14 to 18 days. The greenhouse temperature was 24 ± 2 C; the relative humidity was 65 to 75%. The propagation room was maintained at 24 C and 60% relative humidity with 14 hr of light (13,500 ± 4,000 lux or 1,280 ± 400 ft-c) supplied by fluorescent and incandescent bulbs suspended 90 cm above the leaf surface. Tap water was applied to the soil surface when required. The seedlings were treated before use with demeton (Chemagro, Kansas City) for insect control. Before transplanting, seedlings were carefully separated and the roots trimmed to 5 cm. They were gently agitated in tap water to remove soil, then blotted on paper towels to remove excess water. Each plant used had two or three leaves and weighed 0.1 to 0.2 g fresh.

Fungus and virus culture.—An isolate (No. 69S16-1Fus) of *F. oxysporum* f. sp. *conglutinans* obtained in 1969 from infected field cabbage was established on potato-carrot-dextrose agar slants at room temperature and stored at 2 C. The isolate was re-established on agar once a year, and we checked virulence at that time by passing the fungus twice through the susceptible cabbage Early Marvel in the greenhouse.

Isolates I and II of TuMV were maintained in the greenhouse by manual transfers to turnip (*B. napus* L. 'Laurentian') or *B. campestris* var. *chinensis* seedlings. Unless otherwise indicated, TuMV isolate II was used in all experiments.

Preparation of inoculum.—*Fusarium oxysporum* f. sp. *conglutinans* was cultured in 250 cc of Richard's solution (13) in a 500-cc flask for 18 days at 24 ± 1 C under constant shaking. The mycelia were homogenized in a Waring Blendor for 3 min, then centrifuged at 2,000 *g* for 7 min. The resulting mycelial pellet was washed 3 times in 10 times the original volume of tap water to remove nutrients and metabolic products. The final pellet was suspended in 100 times the original volume of tap water. The final concentration of inoculum was ca. 10^4 propagules/cc of suspension determined by serial dilution on agar plates.

Leaves of TuMV-infected *B. campestris* var. *chinensis* or turnip seedlings harvested 10 to 20 days after inoculation were ground in a mortar with 0.1 M phosphate buffer (pH 8.0) at a volume ratio of ca. 1:1.

Inoculation techniques.—*Brassica campestris* var. *chinensis* seedlings were inoculated with the fungus by root dips in the inoculum suspension. Control seedlings were dipped in tap water.

We made virus inoculations by finger-rubbing the inoculum on Carborundum-dusted leaves of seedlings prior to uprooting from the rearing box. Leaves of control seedlings were rubbed with buffer only.

Disease indexing.—Fungus disease severity was scored from 0 to 5 as follows: 0 = no symptom; 1 = trace of yellowing and very slight or no distortion of the lower leaves and slight to no stunting of the plant; 2 = slight yellowing and distortion of the first and second lower leaves and slight stunting; 3 = moderate yellowing and distortion of first to fourth lower leaves and slight to moderate stunting; 4 = severe yellowing and distortion of all leaves and severe stunting; and 5 = dead plant. All plants in each pot were scored, and the ratings totalled and divided by the number of plants to give a disease index for the pot. The virus disease symptoms were not scored because severity of symptoms on plants inoculated with both virus and fungus was indistinguishable from that on plants infected with virus alone.

The effects of treatments on plant top growth were also compared on a fresh weight basis. For this purpose, each plant was severed at the cotyledonary node.

In some experiments, each stem was dissected and examined for vascular discoloration due to fungus infection. To recover the pathogen, a 3- to 4-mm stem section was removed from each plant above the cotyledonary node and plated aseptically on water agar.

Experimental design.—Each replication consisted of five seedlings transplanted into a 13-cm clay pot containing a mixture of peat moss, sandy loam, and sand. Each pot was plunged to a depth of 10 cm in steamed sand in a 19 X 25 X 15 cm plastic pail. Unless otherwise indicated, there were five replicates/treatment. The pails were placed in a Wisconsin temperature tank at random in blocks in which each treatment occurred once; the water level corresponded to that of the soil surface, and blocks were rotated every 4 days. Room air was held at 20 C and 60% relative humidity. Plants were illuminated as in the propagation room. All experiments were performed twice.

The seedlings were watered as required and fertilized weekly with a 20-20-20 fertilizer solution (5 g/liter) at the rate of 40 cc/pot maintain vigorous growth. Care was taken that water did not splash from pail to pail during watering. Duration of experiments varied from 2 to 5 weeks.

Significant differences between results were determined by Duncan's (4) multiple range and multiple F tests.

RESULTS AND DISCUSSION.—*Simultaneous fungal and viral infections*.—To determine the effect of simultaneous infections of 18-day-old seedlings of *B. campestris* var. *chinensis* by both fungus and virus, plants were inoculated with either (i) *Fusarium* + virus; (ii) *Fusarium* alone; (iii) virus alone; or (iv) no pathogen (control). TuMV isolate I was used as virus inoculum. All treatments were replicated 8 times, and the plants were grown at soil and air temperatures of 28 C and 22 C, respectively. Disease symptoms were recorded weekly, and plants were weighed 5 weeks after inoculation.

Combined infection with TuMV and *F. oxysporum* f. sp. *conglutinans* in *B. campestris* var. *chinensis* increased the severity of cabbage yellows

(Table 1). This effect was observed in the 1st week after inoculation, and persisted throughout the experiment. Plants inoculated with both fungus and virus weighed significantly less after 5 weeks than plants inoculated with each agent alone. The fungus was recovered only from plants showing leaf symptoms of cabbage yellows and vascular discoloration. Only plants deliberately infected with TuMV yielded the virus when indexed on *Nicotiana glutinosa* L. seedlings.

These results showed the same trend as those of Nitzany (9), who reported that cucumber plants inoculated simultaneously with *Pythium ultimum* and cucumber mosaic virus were also more severely diseased than plants inoculated with *Pythium* alone.

Effect of prior viral infection on fungal infection.—The effect of length of virus establishment on subsequent fungus infection was determined on four batches of 14-day-old seedlings virus-inoculated at day 0. A batch was then fungus-inoculated either at day 0, 3, 7, or 15, after which the seedlings were transplanted. Controls consisted of virus-inoculated seedlings and healthy seedlings that were dipped in water and rubbed with Carborundum at day 0; the plants were transplanted at day 0, 3, 7, and 15. After the second (fungus) inoculation, plants were maintained at 25 C (soil) and 22 C (air) temperatures for 16 days. Plants inoculated with both virus and fungus were more severely stunted than plants inoculated with virus alone regardless of the interval between inoculations (Table 2). Fungus and virus were recovered only from the respectively inoculated plants.

TABLE 1. Effect of simultaneous infection of *Brassica campestris* var. *chinensis* with *Fusarium oxysporum* f. sp. *conglutinans* and turnip mosaic virus on severity of cabbage yellows and plant weight

Treatment	Yellows disease index[a] (weeks after inoculation) 1	2	4	5	Fresh wt (g)/plant[b] (5 wk after inoculation)
Fusarium + virus	2.8	4.2	4.1	4.0	3.2 d
Fusarium alone	1.1	2.9	2.8	2.9	7.1 c
Virus alone	0.0	0.0	0.0	0.0	11.3 b
No pathogen (control)	0.0	0.0	0.0	0.0	15.2 a

[a] Disease index 0 to 5, where 0 = no symptoms; 1 = trace of yellowing and very slight or no distortion of the lower leaves and slight to no stunting of the plant; 2 = slight yellowing and distortion of the first and second lower leaves and slight stunting; 3 = moderate yellowing and distortion of first to fourth lower leaves and slight to moderate stunting; 4 = severe yellowing and distortion of all leaves and severe stunting; and 5 = dead plant.

[b] Figures labeled with a common letter are not significantly different at 5% level. Data are means of eight pot replicates, each pot with five plants grown at 28-C soil temperature.

TABLE 2. Effect on weight of *Brassica campestris* var. *chinensis* seedlings by prior turnip mosaic virus infection and subsequent infection by *Fusarium oxysporum* f. sp. *conglutinans*, or by prior infection with *Fusarium* and subsequent virus infection

Treatment			Fresh wt (g)/plant[a]
Virus inoculated before *Fusarium*			
Virus	0[b]	*Fusarium*	0.3 c
Virus alone			2.7 b
No pathogen (control)			6.9 a
Virus	3	*Fusarium*	2.7 c
Virus alone			8.3 b
No pathogen (control)			14.4 a
Virus	7	*Fusarium*	1.4 c
Virus alone			8.8 b
No pathogen (control)			16.5 a
Virus	15	*Fusarium*	7.9 b
Virus alone			12.9 b
No pathogen (control)			26.0 a
Fusarium inoculated before virus			
Fusarium	0	virus	0.8 c
Fusarium alone			2.4 b
No pathogen (control)			7.6 a
Fusarium	3	virus	1.2 c
Fusarium alone			4.8 b
No pathogen (control)			9.5 a
Fusarium	7	virus	4.7 b
Fusarium alone			7.0 b
No pathogen (control)			11.9 a
Fusarium	15	virus	11.0 c
Fusarium alone			15.0 b
No pathogen (control)			17.8 a

[a] Plants from each batch were harvested 16 days after inoculation with the second pathogen. Data are means of five pot replicates, each pot with five plants grown at 25-C soil temperature. Figures within the same batch labeled with a common letter are not significantly different at 5% level.

[b] Inoculation interval (in days) between the first and second pathogen.

Scott (14) observed that oats and wheat inoculated with barley yellow dwarf virus 4 days before inoculation with *Cochliobolus sativus* were consistently more severely affected by root rot than plants infected with the virus or fungus alone. Escobar et al. (5) reported that Pythium root rot of peas was much more severe in plants previously infected with bean yellow mosaic virus or common pea mosaic virus than in fungus-infected plants. Bateman (1) reported that cucumber mosaic virus increased the susceptibility of cucumber plants to attack by *Rhizoctonia* sp., generally on the 3rd or 4th day after virus inoculation. Our data showed that the period of prior establishment of TuMV in *B. campestris* var. *chinensis* did not affect the severity of damage subsequently caused by *F. oxysporum* f. sp. *conglutinans*.

TABLE 3. Effect of temperature on weight of *Brassica campestris* var. *chinensis* due to interaction between *Fusarium oxysporum* f. sp. *conglutinans* and turnip mosaic virus

Treatment	Fresh wt (g)/plant at soil temperature (C)[a]			
	14	21	28	35
Fusarium + virus	2.3 ef	2.3 ef	0.7 g	0.7 g
Fusarium alone	4.5 bc	4.8 b	2.3 ef	1.6 fg
Virus alone	2.5 ef	4.1 bcd	4.9 b	2.8 de
No pathogen (control)	5.2 b	7.1 a	7.9 a	3.4 cde

[a] Data are means of five pot replicates, each pot with five plants. Any two figures labeled with a common letter are not significantly different at 5% level.

Effect of prior fungal infection on viral infection.—To determine the effect of previous fungus establishment on subsequent virus infection in *B. campestris* var. *chinensis*, four batches of 17-day-old seedlings were fungus-inoculated at day 0. A batch was then virus-inoculated either at day 0, 3, 7, or 15, after which the seedlings were transplanted. Controls consisted of fungus-inoculated seedlings and healthy seedlings root-dipped in water at day 0; these plants were transplanted at day 0, 3, 7, and 15. All plants were maintained initially at a 14-C soil temperature to allow establishment of the fungus. After virus inoculation, they were maintained at 25 C soil temperature for 16 days. Severity of cabbage yellows disease was increased by virus infection regardless of the interval between inoculations (Table 2). Weight of plants inoculated with fungus and virus was significantly less than plants inoculated with *Fusarium* alone. Plants inoculated with *Fusarium* alone weighed less at all periods than the healthy controls. Virus was recovered only from inoculated plants.

Effect of temperature on fungus-virus interaction.—Temperature effect on the fungus-virus interaction in *B. campestris* var. *chinensis* was determined on 15-day-old seedlings subjected to four different inoculations: (i) *Fusarium* + virus; (ii) *Fusarium* alone; (iii) virus alone; or (iv) no pathogen (control). Plants were held at 14, 21, 28, and 35 C soil temperatures for 16 days. Interaction occurred on doubly inoculated plants when grown at 21 or 28 C and not at 14 or 35 C (Table 3). Weights of doubly inoculated plants at 14 C were less than those of plants inoculated with *Fusarium* alone, but did not differ from weights of virus-inoculated plants, thus indicating a lack of interaction. However, interaction became evident at 21 C with doubly inoculated plants which weighed less than plants inoculated with either pathogen alone. This effect became more pronounced on plants at 28 C. At 35 C, on the other hand, the interaction was not apparent; weights of plants inoculated with both pathogens were less than plants with virus alone, and not different from plants with *Fusarium* alone. Plants inoculated with both pathogens or with *Fusarium* alone were most severely stunted at 28 and 35 C, whereas plants inoculated with virus alone were most severely stunted at 14 and 35 C. Control plants grew very poorly at 35 C and best at 21 and 28 C.

Various workers (11, 12, 15, 16) have found that temperature affects development of both *F. oxysporum* f. sp. *conglutinans* and TuMV in the host plant. Tisdale (15) found that *F. oxysporum* f. sp. *conglutinans* initiated yellows in cabbage seedlings at soil temperatures ranging from 17 to 35 C, the optimal range for disease development being 26 to 29 C. Walker & Smith (16) reported that severity of the disease produced by *F. oxysporum* f. sp. *conglutinans* in susceptible and resistant cabbage increased with soil temperature to about 28 C, but was retarded at 33 C. Similarly, Pound & Walker (12) showed that the cabbage strain of turnip virus 1 occurred in greater concentration in cabbage grown at 28 C than at 16 C. Pound (11) observed that the virus concentration of cabbage virus A (a strain of TuMV) was low where symptoms were very mild or masked, but high where symptoms were severe and persistent. Our data revealed that temperature affected the severity of disease induced by the combined infection with *F. oxysporum* f. sp. *conglutinans* and TuMV.

The nature of the interaction between a fungus and virus on a given host may indicate the type of disease control required. When a virus enhances the severity of a root disease, control of the virus is relevant. Our data indicate that under favorable conditions, greater reductions in yield of *B. campestris* var. *chinensis* result from the presence in the plant of both *F. oxysporum* f. sp. *conglutinans* and TuMV. So far as the authors are concerned, this apparently is the first report of interaction between systemic fungal and viral pathogens in crucifers. We recommend control of both *F. oxysporum* f. sp. *conglutinans* and TuMV whenever they are encountered in the field. In those areas where yellows-susceptible cultivars are grown, and in fields that have a cabbage yellows history, control of TuMV is extremely important.

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