Induction of Flowering through Infection by Beet Leafhopper Transmitted Virescence Agent

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ABSTRACT

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Circulifer tenellus transmitted the beet leafhopper transmitted virescence agent (BLTVA) to selected plant hosts, which included long-day plants grown under noninductive short days and cold-requiring annuals or biennials grown entirely in a warm greenhouse. Radish and dill infected with BLTVA bloomed. Infected spinach plants did not bloom, but stems grew to an average length of 2.8 cm compared to an average stem length of 1.1 cm in control plants, (different at $P = 0.01$). A high percentage of flowering was observed in infected environmentally noninduced Chinese cabbage, celery, and carrot. Control plants of each species, fed upon by uninoculative insects, did not bloom. These plant responses may be of particular significance since a plant growth substance, gibberellic acid (GA), is known to induce flowering in the specific plants tested. *Catharanthus roseus*, a host species with a GA-insensitive flowering rate, did not bloom prematurely when infected with BLTVA. Applications of two inhibitors of GA biosynthesis, CCC and AMO-1618, delayed the BLTVA-induced host induction response.

Plants infected with mycoplasmalike organisms (MLOs) often exhibit symptoms that might be attributed to a disruption of the host's normal hormone balances. Symptom types have been reviewed (11,13) and include a shortening or lengthening of internodes, reduced rates of growth, a loss of apical dominance resulting in a proliferation of lateral branches known as "witches' brooms," and the abnormal development of floral organs.

Several researchers have explored the hypothesis that the mechanisms of pathogenicity for these organisms might involve a change in the level of some plant growth substance in the infected host. Applications of gibberellic acid (GA) to aster infected with the eastern strain of aster yellows (AY) prevented the stunting effect of the pathogen on plants but had no effect on other symptoms (10). Shepardson and McCrum (14) reported that GA applied as a soil drench to Madagascar periwinkle plants, *Catharanthus roseus* (L.) G. Don, produced symptoms very similar to those observed in plants infected with AY-MLO. Davey and Stodem (5) found that, in *C. roseus* infected with several different strains of MLOs, the levels of cytokinins were increased in flowers but decreased in roots and mature leaves. Most recently, Chang and Donaldson (2,3) proposed that MLO infection reduces kinetin concentration in infected plants which in turn results in phyllody. This effect can be partially prevented by the application of exogenous kinetin.

There is some evidence that the stunting caused by plant pathogenic spiroplasmas, the cultivable mollicutes, is related to changes in host gibberellin metabolism. Maramorosch (10) saw an elongation of corn stems infected with the corn stunt spiroplasma after GA application that was similar to the elongation he observed in AY-infected plants. Daniels (4) investigated the possibility that spiroplasmas interfere with gibberellin metabolism, but the results were inconclusive.

Our recent observations also suggest that changes in host hormone balances are an important part of the physiology of MLO infection. For the past few years, we have been working with an MLO known as beet leafhopper transmitted virescence agent (BLTVA). It is of particular interest because of its possible ecological relationship with *Spiroplasma citri* Saglio et al, the citrus-stubborn agent.

In our efforts to characterize this MLO, we studied the transmission of BLTVA by its only known vector, *Circulifer tenellus* (Baker) (7). We conducted an experiment in winter with a group of about 200 radish plants; the half that were infected with BLTVA bloomed under the noninductive short days of the season, but none of the uninfected plants bloomed although they were held for a full month beyond the time when the majority of infected plants first flowered. This was in contrast to tests done in late summer when both healthy and infected plants bloomed. At that time the most obvious difference between them was the distortion and phyllody of flowers associated with BLTVA infection. These

initial observations led to the work described here.

Gibberellic acid is a plant growth substance known to initiate flowering in some specific groups of plants under certain conditions (15). Generally, GA can substitute for either cold days or long days (LD) in rosette plants, which require one or both conditions for flowering. The flowering of the radish test plants in our initial tests might be explained by the influence of BLTVA on GA-mediated host responses. In this study we tested the possibility that infection by this MLO could induce flowering in a number of different hosts, particularly LD annuals, winter annuals, and biennials selected for their characteristic responsiveness to GA applications. *C. roseus*, a host that is reported to have a GA-insensitive flowering rate (8), was infected with BLTVA as a negative control. In addition, we tested the possibility that CCC ((2-cloroethyl)tri-methylammonium chloride) or AMO-1618 (2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine-carboxylate methyl chloride), both specific inhibitors of GA synthesis, could prevent the effects observed in infected plants.

MATERIALS AND METHODS

Pathogen, insects, and plants. Line FC-83-13 of BLTVA, which has been described and characterized as an MLO (6), was used throughout these tests. It was maintained in a greenhouse in either periwinkle or radish.

The beet leafhoppers, *C. tenellus*, were reared on sugar beet, *Beta vulgaris* L., under conditions reported previously (7). Inoculative leafhoppers were obtained by placing several hundred adults on BLTVA-infected radish, allowing the females to oviposit for 2 wk, removing them, and then waiting 6–8 wk to collect the highly infective adult progeny leafhoppers. High transmission levels of BLTVA were achieved with insects reared in this manner (7).

Greenhouses were fumigated at least biweekly with DDVP (2, 2-diclorovinyl dimethyl phosphate) to prevent any incidental spread of insect-borne phytopathogens. Indicator plants were periodically exposed to leafhoppers to ensure that stock colonies remained free of contamination.

Long-day plants. Dill (*Anethum graveolens* L. 'Burpee's 6144'), radish (*Raphanus sativus* L. 'Scarlet Turnip White Tipped'), and spinach (*Spinacia oleracea* L. 'Melody') were selected because they are known hosts of BLTVA (6) and have been reported to respond to exogenous applications of gibberellin (15). Plants of each species were grown from seed in our greenhouses under the short days of winter or in an environmental chamber set for a 10-hr day. When the plants were about 3 to 4 cm in height, 10 inoculative adult leafhoppers were placed on each plant. At the same time, uninoculative insects were placed on control plants. All plants were fumigated after 1 wk and maintained free of insects for the remainder of the experiment. For 8–10 wk, the plants were examined for the development of flower initials and, at the experiment's conclusion, were measured from the cotyledon leaf scar to the apex of the stem. This experiment was repeated at least once for each species of host plant for a total of not less than 30 infected and 30 control plants of each species.

Cold-requiring annuals and biennials. Young plants of Chinese cabbage (*Brassica rapa* L., Pekinensis Group 'Michihli'), carrot (*Daucus carota* L. var. *sativus* 'Red Cored Chantenay'), and celery (*Apium graveolens* L. var. *dulce* 'Fordhook') were exposed to inoculative leafhoppers in early spring and allowed to grow throughout the long days of late spring and summer. These test species are hosts of BLVTA (6) and are normally induced to flower when vernalization is followed by long days (15). These experiments were repeated twice involving a total of at least 20 inoculated and 20 uninoculated plants of each species. The plants were observed for at least three months and examined frequently for the development of flower initials, or the lack of initials.

GA-insensitive host. *C. roseus*, a tropical plant frequently used as an indicator host for MLOs, was selected as a negative control because its flowering rate is not affected by the application of GA (8). Young periwinkle plants ('Little Pinkie') were given three treatments: exposure to BLTVA inoculative leafhoppers, exposure to uninoculative leafhoppers, and exposure to uninoculative leafhoppers followed by treatment with GA. GA was applied by placing 5 drops of a 100 μg/ml solution on the plant apex 2 and 3 wk after the insects were removed. The experiment was repeated twice for a total of 24 plants in each treatment group. The day of first flowering was recorded for each test plant.

Inhibitor treatment. The inhibitors CCC and AMO-1618 have been shown to delay GA-mediated responses in treated plants. To determine their ability to delay the BLTVA-associated induction of flowering, greenhouse-grown radish plants were inoculated in the spring or fall and then treated with both inhibitors. Control plants were exposed to uninoculative leafhoppers at this time. Our preliminary experiments had indicated that, although uninfected radish plants may flower during spring and fall, they do so far less quickly than infected or GA-treated plants. In turn, the infected and GA-treated plants respond in less time than they might during short days.

CCC was applied to BLTVA-inoculated and uninoculated control plants as a root drench of 100 ml of a 2 g/L solution twice weekly. Postinoculation treatments were applied for 5 wk. AMO-1618 was applied to plants as a 100-ml-per-pot root drench of a 0.05 g/L solution once a week for 5 wk.

Weekly observations were made of each plant, and height measurements and degree of development were noted. Development was recorded as rosette (leaves radiating from a crown where internodes are extremely short), bolting (stem elongation without or before flowering), or flowering (buds visible at the shoot apex). These experiments were repeated for a total of at least 25 plants in each treatment group.

RESULTS

Long-day plants. Seven weeks after inoculation with BLTVA, 35 of 42 dill plants were flowering, whereas only 2 of 41 plants exposed to uninoculative leafhoppers were flowering. The inoculated plants that flowered had symptoms typical of BLTVA infection including floral virescence, floral proliferation, internode elongation, and foliar yellowing. The control plants that bloomed appeared normal. In one experiment, dill plants were kept for an additional 4 wk; by that time the healthy plants also had flowered. Average height of the 16 BLTVA-infected plants as measured from the soil line to the top of the umbel was 107.7 cm, while the average height of 17 healthy plants was only 83.1 cm (different at $P = 0.01$).

Of 45 radish plants inoculated with BLTVA, 38 had bloomed with symptomatic virescent flowers after 10 wk. Only 2 of 45 control radish plants had flowered at this time. Flowers on these two plants were normal. Unlike the dill plants, infected radish plants were about the same height as control plants.

Healthy spinach grown under short days remained in a nonflowering rosette stage. Although BLTVA-inoculated spinach did not flower, it did bolt. Stems were visibly elongate on the inoculated test plants. Infected plants had an average stem length of 2.8 cm for 11 plants, while the average for the same number of healthy plants was only 1.1 cm (different at $P = 0.01$).

Cold-requiring annuals and biennials. Chinese cabbage responded uniformly to infection by blooming within 4 mo. All of 27 inoculated plants flowered, exhibiting typical BLTVA symptoms, while 21 control plants grew as tight, compact heads (Fig. 1). The response of the biennials tested was equally pronounced. Of 33 inoculated celery plants, 32 bloomed. None of the 21 control celery plants bloomed. Although we have grown celery for more than 10 years in our greenhouses as a leafhopper host plant, this was the first time celery had bolted under our rearing conditions. In carrots, 11 of 18 inoculated plants bloomed, while none of the 18 control plants flowered within 4 mo.

GA-insensitive host. All periwinkle plants flowered within the same 1-wk period regardless of treatment. Although the GA-treated plants were taller than the BLTVA-infected and healthy plants, there were no differences in flowering rate.

Inhibitor treatments. CCC reduced flowering 54% in treated plants at 6 wk. However, within 8 wk, all infected plants, whether inhibitor treated or not, were flowering.

AMO-1618 also reduced the flowering response produced by BLTVA infection in radish. At 6 wk, AMO-treated plants were flowering at nearly half the rate of the water-treated BLTVA-infected plants (Fig. 2). Sixteen percent of the healthy plants were blooming under the environmental conditions of the experiment, and the flowering rate of uninoculated plants was also reduced by AMO-1618 treatment. Again by 8 wk the inhibitor-treated plants had reached the flowering rate of the water-treated infected plants.

DISCUSSION

Dill, radish, and spinach normally flower under long days. Under short days, if GA is applied, dill and radish will flower. Spinach responds with stem elongation but does not bloom. In the case of Chinese cabbage, carrot, and celery, vernalization followed by long days induces flowering. Exogenous GA application is known to substitute for the cold requirement, and flowering should occur subsequently if long days are provided. Flowering rate of periwinkle is not affected by applications of exogenous GA.

Table 1 lists the percentages of plants of each species flowering at the end of experimental trials. There is an absolute correlation between the expected response relative to flowering rate of a given species to exogenously applied GA and its actual response to infection with BLTVA. Those inoculated radish and dill that had not flowered when the data were taken were held for an additional period until flowering occurred. All of these plants had normal flowers. Therefore, variation in the percent of infected plants flowering from species to species may represent a difference in rate of infection rather than response to infection. In the case of the Chinese cabbage, carrots, and celery, further work should include an assay for infection. Since the only diagnostic symptoms in these hosts are floral (6), plants that have been inoculated but do not flower could be vernalized and held until the presence or absence of floral symptoms could be observed.

In addition to the host induction response (HIR), which is defined as the ability of BLTVA to induce flowering in host plants grown in noninductive environmental conditions, internode elongation was observed in both dill and spinach. It has also been observed in a number of other BLTVA hosts (6). Although GA is a well-known hormone that can cause stem or internode elongation, other plant growth substances can also do the same. Therefore, while stem elongation is a suggestive symptom in infected plants, which may result from altered GA metabolism, it is not as specific as the HIR. In addition, the absolute length of any internode will be the sum of all factors affecting the host; the lengthening brought about in some hosts, perhaps as a result of raised GA levels, might be obscured by the stunting effects of disease.

The correlation of incidence of HIR with those plants that respond in a like fashion to exogenous GA application, the

TABLE 1. Flowering in plants inoculated with the beet leafhopper transmitted virescence agent and in their uninoculated counterparts

Host plant	Inoculated flowering (%)	Control flowering (%)	GA-sensitive flowering rate
Long-day plants			
dill	83	5	+
radish	84	4	+
spinach	0	0	−
Cold-requiring plants			
Chinese cabbage	100	0	+
carrot	61	0	+
celery	96	0	+
Other			
periwinkle	100	100	−

Fig. 1. Chinese cabbage, *Brassica rapa* L. 'Michihli,' bloomed 12 wk after inoculation with the beet leafhopper transmitted virescence agent even though plants did not receive the vernalization normally required to induce flowering. The control plants (left), which were not exposed to the agent, grew as compact heads.

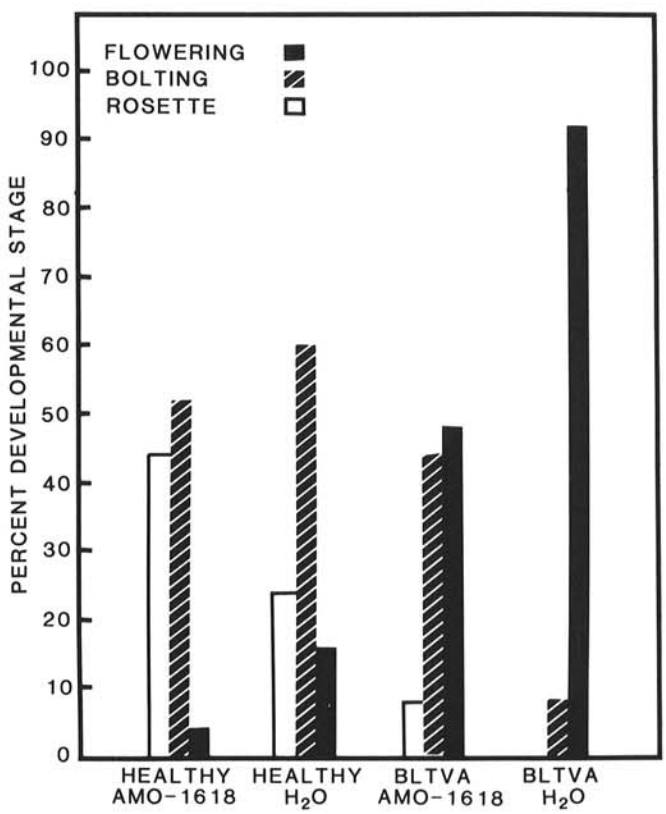

Fig. 2. Effect of the gibberellic acid biosynthesis inhibitor AMO-1618 upon the percentage of radish plants, *Raphanus sativus* L. 'Scarlet Turnip White Tipped,' that bolted or flowered 6 wk after exposure to *Circulifer tenellus* inoculative with the beet leafhopper transmitted virescence agent. Healthy plants were exposed to uninoculative leafhoppers.

inability of BLTVA to stimulate a similar response in spinach and periwinkle (plants with a flowering rate insensitive to applications of GA), and the ability of two inhibitors of GA synthesis, AMO-1618 and CCC, to reduce the rate of response of radish plants to infection suggest that the mechanism of the HIR associated with BLTVA infection involves GA metabolism. Because of the serious technical problems involved in either culturing or isolating this agent, determining whether host or pathogen are directly involved in increased synthesis will be difficult. In either case, this agent contains some potentially valuable genetic material; it could be either a source of genes that affect the expression of host plant hormone metabolism or a source of prokaryotic DNA coding for genes involved in the synthesis of gibberellins.

The ability to induce HIR in infected hosts could be investigated with other MLOs. The distribution of this trait could be suggestive of taxonomic relationships within the group. There are some indications that this character might be found in other MLOs. Bos and Grancini (1) in the 1960s mentioned that clover phyllody causes premature flowering of carrot. Hooper et al (9) reported the premature sprouting of MLO-infected onions, and Martin (12) has observed that GA shortens the rest period of some root crops. Perhaps the widespread use of *C. roseus* as an indicator plant by workers in this field may have prevented them from observing this trait. *C. roseus* is a convenient and reliable indicator plant for MLOs because it can be vegetatively propagated by grafting or cuttings, thus avoiding the need for time-consuming vector passages. However, as our work demonstrates, the BLTVA HIR is masked in periwinkle. Therefore, it is possible that the ability of any individual MLO line to induce HIR could go unnoticed in periwinkle for many years.

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