Effect Of Green Manure Crops and Organic Amendments on Incidence of Nematode-Borne Tobacco Rattle Virus

Frans C. Zoon[2], Anne Sophie van Bruggen[1], Ate de Heij[2] , Cees J. Asjes[1]
and J.E. van den Ende[1]
[1] Bulb Research Centre
P.O. Box 85, 2160 AB Lisse
The Netherlands.
Tel. +31-252-462121
Fax +31-252-417762
e-mail: anne-sophie.van.bruggen@lbo.agro.nl

[2] Plant Research International
P.O. Box 16, 6700 AA Wageningen
The Netherlands
Tel.+31-317-476000
Fax +31-317-410113

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Abstract

Tobacco rattle tobravirus (TRV) may infect several ornamental bulb crops and is transmitted by trichodorid nematodes. *Paratrichodorus teres, P. pachydermus* and *Trichodorus similis* are the main vectors in the Netherlands. In field experiments the effects of various pre-crops and organic amendments on the TRV Infection Potential of Soils (TRV-IPS) and on disease level in tulip and gladiolus were studied. Organic matter amendment of soil at a rate of 1% dry weight has been shown to reduce the host finding activity of *P. teres* under laboratory conditions. In a field containing viruliferous *P. teres* dahlia, italian ryegrass, white mustard and fodder radish were grown or the soil was kept fallow and the resulting TRV-IPS prior to the bulbous test crops was measured by a soil dilution bait test method. The application of organic matter was tested after dahlia as pre-crop. Household waste compost (GFT compost) was applied as a soil mix or planting furrow treatment at 12 tons dry weight per ha for tulip and gladiolus. Spent mushroom compost (Champost) was added as planting furrow treatment at 17 or 12 tons dw/ha, respectively, for tulip and gladiolus. The percentage of TRV diseased plants was determined at flowering in all pre-crop and organic amendment treatments. Champost in the planting furrow and fodder radish as a preceding crop reduced the percentage infection in tulip under favourable conditions for TRV infection. In gladiolus most organic amendments, fodder radish as pre-crop and keeping the soil fallow reduced the TRV infection rate of the plants during the first growing season, but not of the plants grown from the corms in the next year.

INTRODUCTION

Tobacco rattle tobravirus (TRV) is transmitted by trichodorid nematodes (*Trichodorus* and *Paratrichodorus* spp.: Trichodoridae) and infects several bulbous and perennial ornamental crops, causing growth reduction and quality loss due to specific symptoms, such as necrotic lesions on leaves, plant malformation or flower colour breaking (Asjes, 1989). This virus-vector complex also causes disease in potato (*Solanum tuberosum*), with growth reduction, stem mottle and corky ring necrosis (spraing) in the tubers, as well as in pepper (*Capsicum annuum*) with chlorotic rings on leaves and malformed fruits (Taylor and Brown, 1997). The disease is called rattle (ratel) in tulip, notched leaf (kartelblad) in gladiolus, and malaria in hyacinth. Symptoms can occur during the season in which infection took place (primary infection), but also in the next year (secondary infection). In gladiolus primary infection mainly is the result of transmission of TRV into the tissue of young sprouts emerging from the corm. Secondary infection mainly is caused by viruliferous nematodes attacking the roots (Cremer and Schenk, 1967). Very low incidence of infection already disqualifies a field of bulbs from certification, causing serious economic loss.

Trichodorid vector nematodes occur in sandy soils (clay 1-15%, *Trichodorus primitivus* up to 25 %). They have a very wide host range and, apart from being virus vectors, they may also cause direct damage to seedlings of many crops and below ground parts of the stems of potato. In The Netherlands, *Paratrichodorus teres*, *P. pachydermus* and *Trichodorus similis* are the major vectors of TRV in flowerbulb growing areas (Ploeg et al., 1991). These vector species transmit serologically distinct isolates of TRV (Ploeg et al., 1992). Host finding by *P. teres* appears to be very effective. In conducive soils Trichodoridae may find seedlings from more than 10 cm away, especially when plant signals are transported downwards by water infiltration (Jones, 1975; Zoon and Maas, 1996).

Two strategies are explored for the prevention of TRV infection in tulip and gladiolus: 1. reduction of the TRV-IPS, *viz.* the number of viruliferous vectors, and 2. (temporary) reduction of the infective activity, *viz.* host finding of the vectors. Until now, these aims were often achieved by the use of soil fumigation and granular nematicides (Maas, 1974), which are under pressure of regulation and restriction of use. As trichodorid vector nematodes and TRV both have a wide host range, it is a challenge to find crops which are non-host for the vector as well as for the virus, and which can be implemented in rotations with flower bulbs in order to reduce the TRV-IPS. In greenhouse experiments, fodder radish (*Raphanus sativus*) was found to be a non-host for TRV and different trichodorid vector species (Zoon and Asjes, 1996; Zoon and Maas, 1996). Vector activity might be inhibited temporary by organic amendments (Zoon and Maas, 1996; De Heij and Zoon, 1996) or constitutively by increasing the clay content of the soil (Kuiper, 1977). The latter seems a rather difficult way to achieve sufficient suppression of host finding. In this paper we report on field experiments with *P. teres* in the search for vector- and virus-resistant pre-crops and organic amendments for environment-friendly prevention of TRV infection in tulip and gladiolus.

MATERIALS AND METHODS

Field Experiment 1: Effects of Pre-Crops and Organic Amendments in Tulip (*P. teres*)

An infested field was homogenised and on small plots (netto 1 x 6 m) different crops were grown during 10 weeks in summer. In addition to the fallow control there were Dahlia (*Dahlia variabilis;* 'Mignon'; seedlings), white mustard (*Sinapis alba;* 'Maxi'), fodder radish (*Raphanus sativus*; 'Nemex'), and italian ryegrass (*Lolium multiflorum*; 'Tetila'). Before planting of tulips (*cv*. 'Apeldoorn') late September, crops were mown and ploughed in. Soil samples were taken from each plot for estimation of the TRV infection potential of the soil (TRV-IPS; De Heij et al., 2000). Organic amendments were applied after dahlia just before planting of the bulbs at maximum allowed rates as indicated in table 1. Organic amendments were tested in the plots were dahlia had grown, because it has been known that after this crop a high TRV-IPS is present. Fresh household waste (GFT) compost was kept 4 weeks before use. Spent mushroom compost (Champost) was obtained from the grower directly after pasteurization and kept for a few days before use. Six hundred tulips were planted per plot. Tulips were treated according to common practice. At flowering 300 of the plants were scored for TRV symptoms on leaves and flowers. The number of tulip plants per 12 m row was counted to determine whether possible phytotoxic effects resulting in loss of plants occurred.

Field Experiment 2: Effects of pre-Crops and Organic Amendments Gladiolus (*P. teres*)

The pre-crops and fallow were the same as in the tulip experiment described above, but the growth period lasted from summer to over winter (only italian ryegrass survived the frost to some extent). Sampling for TRV-IPS assays was done in March and the remaining crops were ploughed in just before planting of gladiolus in April. Organic amendments were applied just before planting of the corms at maximum allowed rates after a culture of dahlia as indicated in table 1. Organic amendments were tested in the plots were dahlia had grown, because it has been known that after this crop a high TRV-IPS is present. Six hundred corms were planted per plot (netto 1 x 6 m). Gladiolus was treated according to common practice. Observation of primary infection (mainly necrotic leaf symptoms and malformation) was done in 300 plants per plot at flowering. Infected plants were removed. Secondary infection was assessed the next year in plantings of harvested corms.

Statistical Analyses

The TRV-IPS was transformed to ln (x+1) for analysis. The percentage of infected plants was transformed to arcsin(sqrt(x/100) for analysis. Data were analyzed for significant differences using LSD (Least Significant Difference) at p=0.05.

RESULTS AND DISCUSSION

Field Experiment 1: Effects of Pre-Crops and Organic Amendments in Tulip (*P. teres*)

Despite the short growing period of the pre-crops, fodder radish caused a decrease and dahlia an increase in the TRV infection potential of the soil (TRV-IPS) resulting in a significant difference between the two (Table 2). The incidence of TRV in tulip was extremely high in this experiment, probably caused by the early planting date (Asjes 1989; Van Hoof, 1975) and favourable conditions. The pre-crop of fodder radish reduced the incidence, which is consistent with the reduced TRV-IPS. After dahlia in combination with champost the incidence was lower as well. This is not due to a lower TRV-IPS, but most likely to inhibition of vector activity or a lower post planting survival. Champost in the planting furrow caused some fytotoxicity. The final number of plants per 12 m row was reduced by a non-significant 3 % compared to the dahlia control. The treatments with GFT-compost were not effective in tulip.

Field Experiment 2: Effects of Pre-Crops and Organic Amendments in Gladiolus (*P. teres*)

The pre-crop of white mustard resulted in the highest TRV-IPS and primary disease incidence (Table 3), but disease incidence after italian ryegrass and dahlia was not significantly lower. Surprisingly, the secondary infection was not affected by the pre-crop. Presumably, a stronger decrease in TRV-IPS, also in deeper soil layers, is needed to achieve effects in the secondary TRV disease in gladiolus as we experienced earlier (Asjes et al., 1997; Asjes et al., 1998).

GFT compost mixed through the entire tilth layer and GFT compost or Champost in the planting furrow reduced the primary infection incidence in gladiolus (Table 4). This indicates a lower efficiency of infection of the young sprouts (Cremer and Schenk, 1967), either due to lower post-planting survival or lower activity of the vector nematodes. GFT in a layer at 20 cm depth was not effective. First of all the compost was not near the corms in this treatment, and secondly, the turning of the soil necessary to obtain the layer may have brought nematode concentrations from deeper layers close to the sprouts. Unfortunately, secondary infection was not reduced by the compost treatments or pre-crops. Apparently, the roots were not sufficiently protected during their extensive susceptible period, nor over their whole depth of occurrence.

CONCLUDING

TRV-incidence in tulip on a field infested with *P. teres* was reduced by either a pre-crop of vector- and TRV-resistant fodder radish, or by organic amendments with champost in the furrow at planting. Champost amendment may cause some phytotoxicity when applied at maximum rates close to the plants. This might be solved by shallow mixing champost and soil before planting bulbs on top of it. The levels of reduction by pre-crops and champost treatments on their own may not be sufficient under conditions favourable for TRV infection as in our field experiments. However, consistently growing resistant crops in the rotation and combining pre-crops and organic amendments with late planting is likely to considerably reduce the risk of TRV infection in tulip. In gladiolus, organic amendments resulted in a reduction of primary infection. On the other hand no effects were observed on secondary infection. For the culture of TRV-susceptible gladiolus cultivars a non-host crop rotation with stringent weed control, and field selection based on soil sampling and TRV detection seem the only options to prevent TRV infection in an environment-friendly way.

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Tables

Table 1. Organic amendments at planting of tulip and gladiolus in field experiments.

Treatment	Amount in Tulip x 1000 kg dry m./ha	Amount in Gladiolus x 1000 kg dry m./ha
Non-amended control	0	0
GFT compost in layer at 20 cm depth	12	12
GFT compost in planting furrow	12	12
GFT compost mix 0-25 cm depth	12	12
Champost in planting furrow	17	12

Table 2. TRV-infection potential of soil and TRV-incidence in tulip as influenced by preceding crops and organic amendments.

Pre-crop july-sept	Amendment at planting	TRV-IPS /400 ml at planting [1]	TRV-inf. % at flowering [2]	# plants per 12 m row
fallow	none	4.3 ab	94 c	344 acbd
fodder radish	none	2.6 a	69 ab	347 abcd
white mustard	none	6.7 ab	98 c	337 cd
italian ryegrass	none	4.7 ab	90 bc	354 abc
dahlia	none	7.2 b	97 c	342 bcd
dahlia	12 t GFT-comp. layer	9.4 b	98 c	355 ab
dahlia	12 t GFT-comp. mixed	9.5 b	96 c	348 bcd
dahlia	12 t GFT-comp. furrow	7.8 b	97 c	361 a
dahlia	12 t Champost furrow	11.8 b	67 a	330 d

[1]. LSD (0.05) and back-transformed means after ln(x+1) transformation; [2]. idem after arcsin(sqrt(x/100)) transformation

Tabel 3. Effects of pre-crop on TRV infection potential of soil (TRV-IPS) and disease incidence in Gladiolus cv. 'Peter Pears'.

Pre-crop treatment	TRV-IPS per 400 ml [1]		% primary TRV-incidence[2]		% secondary. TRV-incidence[2]	
White mustard	5.6	a	11.8	a	21.0	ns
Dahlia	2.0	b	9.9	a	19.1	
Italian ryegrass	1.0	b	9.5	a	14.8	
Fodder radish	0.6	b	4.1	b	24.1	
Fallow	0.5	b	4.1	b	16.2	

[1] Infective units in soil; after ln(x+1)-transformation;
[2] Plants with symptoms; LSD(0.05) and backtransformed means after arcsin(sqrt(x/100))-transformation

Table 4. Effect of organic amendments at planting on TRV infection in Gladiolus.

Compost treatment	TRV-IPS per 400 ml [1]		% primary TRV-incidence[2]		% secondary. TRV-incidence[2]	
None	2.0	ns	9.9	a	19.1	ns
GFT compost layer at 20 cm	1.8		11.4	a	23.7	
GFT compost furrow	1.5		4.6	b	20.6	
GFT compost mix	1.3		3.6	b	17.0	
Champost furrow	2.0		2.7	b	16.3	

[1] Infective units in soil; LSD(0.05) and backtransformed means after ln(x)-transformation;
[2] Plants with symptoms; LSD(0.05) and backtransformed means after arcsin(sqrt(x/100))-transformation