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VERTICILLIUM ALBO-ATRUM AS A PARASITE OF *SENECIO VULGARIS*[1]

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Many weeds, including *Senecio vulgaris*, are virtually symptomless hosts of *Verticillium albo-atrum.* Plants of *Senecio vulgaris* were inoculated with spore suspensions of *V. albo-atrum* by two different methods: root-dipping or injection. Infected plants rarely wilted but were stunted, their lower leaves died prematurely and numbers of lateral shoots were decreased. *V. albo-atrum* was isolated from all parts of the host including achenes. When placed on agar, infected achenes germinated more rapidly than healthy ones. Only small amounts of mycelium developed in the vessels. Severe wilting was possibly avoided because there is insufficient time for damaging amounts of mycelium to develop in *Senecio* which has a short period of vegetative growth. On the other hand its tolerance to infection may be attributed to the morphology of its vascular system with its narrow vessels and sparse parenchyma cells.

INTRODUCTION

In fields, where crop plants were previously infected by *Verticillium albo-atrum* Reinke & Berth., this fungus can readily be isolated from a number of weeds, although they appear to be relatively healthy. VAN DER MEER (1925) isolated *V. albo-atrum* from *Chenopodium album, Chrysanthemum leucanthemum, Erigeron canadense, Senecio vulgaris, Solanum nigrum* and *Urtica urens;* HARRIS (1956) isolated it from *Chenopodium album* and groundsel, *Senecio vulgaris.* In the latter plant the pathogen invaded aerial tissues without causing well defined macroscopic symptoms. VAN DEN ENDE (1956, 1958) added *Fragaria vesca* to the list of possible hosts for *V albo-atrum* and VAN GEEST (1959, unpublished report) isolated the fungus from *Capsella bursa pastoris* and *Oxalis stricta.* A host index of *V. albo-atrum* is given by ENGELHARD (1957).

RUDOLPH (1931) paid attention to disease symptoms developing when *Taraxacum officinale* and *Senecio vulgaris* were attacked. The latter showed the typical syndromal characteristics caused by *V. albo-atrum.* In contrast VAN DEN ENDE found that *Senecio vulgaris* was a virtually symptomless host, like *Chrysanthemum leucanthemum* and *Solanum nigrum,* although RICHTER and OVERDULVE (1962, unpublished reports) observed yellowing and red discoloration of the leaves combined with slight stunting.

Various investigators have reported conflicting observations on the dissemination of *Verticillium* spp. on seeds of several host plants. The fungus may or may not be transmitted on seeds of tomato, eggplant and cotton. SACKSTON & MARTENS (1959) found *V. albo-atrum* abundantly in the hull and even on the testa of seeds from diseased sunflowers, and discussed the conflicting data. Seeds may be contaminated superficially or the fungus may enter them through

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the vascular tissues of the diseased parent plants during the process of ripening. The latter seemed to be the case with wilted safflower plants, *Carthamus tinctorius,* in the field. SCHUSTER & NULAND (1960) found 9% seed infection in field crops and up to 44% in plants grown in infested glasshouse soils. VAN GEEST and OVERDULVE have both isolated *V. albo-atrum* from the achenes of *Senecio vulgaris* and in one instance from the pappus.

The present investigation was done to test further the transmission of *V. albo-atrum* by achenes of diseased *Senecio vulgaris,* and also to study the behaviour of the fungus in this tolerant host. It is remarkable that strains of *V. albo-atrum,* highly virulent on cultivated plants such as tomato and *Antirrhinum majus,* do not cause weeds to wilt. Special attention was paid to the quantity of mycelium present in the host plant, its distribution and the alterations which are induced in the vessels. *Senecio vulgaris* was chosen as a nearly symptomless host and *Balsamina impatiens* as a control, since this species is highly susceptible and readily shows symptoms after inoculation with *V. albo-atrum.*

MATERIAL AND METHODS

Plants were grown in garden soil in a glasshouse at 21° to 25°C and batches of uniform size, with 10 to 15 replicates, were selected for inoculation with a virulent strain, V 1350, of *V. albo-atrum* isolated from strawberry by VAN DEN ENDE. Two inoculation methods were used: a. Root-dipping: Roots were washed free of as much soil as possible and then immersed in a homogenized suspension of a shake culture of *V. albo-atrum* grown in Czapek-Dox solution. Each inoculated plant was subsequently potted separately. b. Injection: 0.1 ml of a spore suspension, containing about 20,000 to 30,000 spores per ml, was injected into the base of each plant.

The presence of the fungus within the host was checked by microscopic examination and isolation. Stem slices, 3 tot 4 mm thick, were immersed in 96% ethanol, flamed and placed on cherry agar. After three to four days incubation mycelium of *Verticillium,* if present, could be readily detected growing superficially. Achenes were surface sterilised in a 2% sodium hypochlorite solution for 2 minutes before being put on agar. For microscopic examination freehand sections were sometimes stained with cotton blue.

RESULTS

Senecio plants were inoculated when 20 cm high by either the root-dipping or injection methods. Within 16 days the infected plants were slightly stunted and had fewer laterals, but there were no other visual symptoms of disease. In spite of the comparative lack of external, and also of internal symptoms, *V. albo-atrum* was isolated from 6 of 10 plants treated by the root-dipping method and from a similar proportion of injected plants (Table 1). The height of the infected plants as well as the average number of their laterals was less than that of the control-plants. Mycelium was observed in longitudinal sections: long straight hyphae running along the walls inside xylem vessels. In comparable tests with *Balsamina impatiens, V. albo-atrum* caused blackening of the vascular system in stem and petioles within 7 to 10 days of inoculation.

When smaller plants of *Senecio,* 15 cm high, were inoculated by root-dipping,

TABLE 1. Effects of different inoculation procedures on the development of disease symptoms in *Senecio vulgaris* caused by *Verticillium albo-atrum* (16 days after inoculation).

Methods of inoculation	*Verticillium* isolated[1]	Mycelium in vessels[1]	Average height in cm of 10 plants[2]	Average number of laterals of 10 plants	Other symptoms
Injection	6/10	2/3	26	2.5	none
Root dipping	6/10	1/2	25	2.6	none
Control	0/10	0/3	28	3.2	none

[1] Numerator: number of plants with *Verticillium*.
Denominator: number of plants examined.
[2] The host plants were 20 cm high when inoculated.

stunting occurred within 10 days. After 14 days the average height of the 15 inoculated plants was 74% of that of the controls; after 21 days, it was only 68% (Table 2, Fig. 1). Other external disease symptoms also appeared: basal leaves of the inoculated plants showed a slight reversible wilting during daytime when temperatures were high, and a red foliar discoloration – the precursor of yellowing and necrosis. These effects on vegetative growth were associated with delayed flowering, and decreased and retarded seed production.

TABLE 2. Development of disease symptoms with increasing time after inoculating 15 plants of *Senecio vulgaris*, 15 cm high, with *Verticillium albo-atrum*.

Number of days after inoculation	Average height		Foliar symptoms[1]	Presence (+) or absence (–) of vascular discoloration in leaf petioles	Isolation of *Verticillium*[2]
	in cm	in % of control			
14	20.5	74	+	–	
21	28.0	68	++	+	3/3
control					
14	27.6	100	–	–	
21	41.2	100	–	–	0/3

[1] + Wilting ++ Wilting and discoloration.
[2] Numerator: number of plants with *Verticillium*.
Denominator: number of plants examined.

Attempts to isolate the fungus from inoculated plants were usually successful. *V. albo-atrum* could be isolated from slices of root, main stem and laterals – it appeared to pervade the entire plant including achenes. The percentage of achenes infected increased with time after inoculating the parent plant (Table 3). After three weeks 20% were infected whereas after five weeks the proportion increased to 68%. Germination of achenes produced by diseased plants was unimpaired, and no correlation was found between infection and abortion. In other tests, achenes harvested from plants, 32 days after inoculation, germinated about one day earlier, on wet filter paper or water agar, than those harvested from healthy plants (Table 4).

Before lower leaves of inoculated plants were discoloured, microscopic examination of stems of inoculated plants showed the presence of only a small quantity of mycelium scattered in the xylem vessels. Later, when discoloration

FIG. 1. *Senecio vulgaris*. Left: control plants.
Right: plants inoculated with *Verticillium albo-atrum*.

and yellowing of the basal leaves began to appear, mycelium was found in larger quantities, forming bundles and clusters partially plugging the vessels. Other internal symptoms such as browning of the vessel walls and gummosis appeared only at the base of petioles of discolored leaves.

Symptoms of *Verticillium* wilt were reproduced when sprouts of *Balsamina* and *Senecio* were kept in culture filtrates of *V. albo-atrum*. As before, *Senecio* seemed less sensitive than *Balsamina*, and symptoms developed more slowly and less severely.

TABLE 3. Percentage of achenes with *Verticillium albo-atrum* harvested from infected plants of *Senecio vulgaris*. At least 100 achenes examined on each occasion.

	Number of days after inoculating parent plants				
	21	23	26	29	33
Achenes sampled from inoculated plants	20	21	26	36	68
Achenes sampled from control plants	0	0	0	0	0

TABLE 4. Comparison of the germination of *Senecio* achenes taken from healthy and *Verticillium* infected plants. The latter were sampled 29 days after inoculation.

Achenes from:	Cumulative totals of % germination of achenes; number of days after sowing		
	2	3	4
Inoculated plants	11	31	97
Control plants	0	27	96

DISCUSSION

The main symptoms induced in *Senecio vulgaris* by *V. albo-atrum* are size decreases and reversible wilting of the basal leaves at high temperature followed by discoloration, yellowing and foliar necrosis. Plant age at inoculation greatly affects the severity of symptoms but even comparatively severely infected plants are able to flower and produce achenes. These have a higher germinative power than those of uninoculated controls.

The tolerance of *Senecio* to infection is probably associated with its short vegetative period. In the field, where the inoculation potential is probably lower than in the experiments described, plants may escape heavy damage. When vegetative growth ceases and achenes mature only the vascular system in the basal sections of petioles of lower leaves will (a) be plugged by mycelium and (b) show browning of vessel walls. The tolerance of *Senecio vulgaris* contrasts with the behaviour of infected *Balsamina impatiens* plants, where vessel walls rapidly brown and plugs form within a few days after inoculation.

It might be that wilting does not occur in *Senecio* as long as the mycelium is only present in small quantities. Because of its short vegetative period, the amount of toxin produced by *V. albo-atrum* in *Senecio* may not reach critical levels – a disease escape mechanism.

KLING (1938) and GRIGORJAN (1949, ref. in SUCHORUKOW, 1958) found a correlation between the anatomy of cotton varieties and their susceptibility to *Verticillium* wilt. Those with a dense xylem and narrow vessels are more resistant than varieties with wide vessels. A similar difference exists between *Balsamina* and *Senecio*. In the susceptible *Balsamina* each vascular bundle includes big vessels surrounded by one or more layers of parenchyma. In *Senecio* the vascular bundles contain a great number of small vessels and tracheids, hardly separated by parenchyma. In *Balsamina* there are a greater number of living parenchyma cells in the xylem which may be damaged by pectic or cellulolytic enzymes produced by the fungus. Oxidation of the phenols and polymerisation of the resulting quinones or other processes may occur in those cells. Vivotoxins in the sense of DIMOND & WAGGONER (1953) may start a chain of reactions resulting in plugging and discoloration of the vessels. A high number of living cells in the xylem would correlate with violent reactions. This is in agreement with the investigations of SUCHORUKOW (1958), who considers the toxin produced by the fungus to be activator of oxidation processes in the living cells adjacent to the vessels, leading to melanin-formation. Finally these parenchyma cells die off.

Although diseased plants of *Senecio* may be stunted and their lower leaves yellow prematurely, they are nevertheless difficult to distinguish in the field from healthy plants. Symptoms are never severe. The tolerance of *Senecio* and other weeds to *Verticillium* provides the fungus with a ready means of survival, in the absence of susceptible crop plants, in which it is not subjected to the antagonism of soil inhabitants. Moreover, by infesting seed without impairing germination, *V. albo-atrum* has developed an ideal method of dispersion to new sites.

SAMENVATTING

Het is gebleken, dat vele met *Verticillium albo-atrum* geïnfecteerde onkruiden geen of slechts zwakke ziekte-symptomen vertonen. Planten van *Senecio vulgaris* werden met *V. albo-atrum* geïnoculeerd door de wortels in een sporensuspensie te dompelen of door de planten met een sporensuspensie te injecteren. De geïnfecteerde planten verwelkten zelden, maar vergeleken bij de controleplanten bleven zij achter in groei; de onderste bladeren stierven voortijdig af en er ontwikkelden zich minder zijspruiten. *V. albo-atrum* kon uit alle delen van de zieke planten geïsoleerd worden, zelfs uit de dopvruchtjes. Indien de laatste na uitwendige ontsmetting op wateragar uitgelegd werden, ontkiemden zij eerder dan niet besmette vruchtjes.

In de vaten van de geïnfecteerde planten ontwikkelde zich slechts een geringe hoeveelheid mycelium. Het uitblijven van verwelkingsverschijnselen kan waarschijnlijk verklaard worden door de korte levensduur van de planten, waardoor het mycelium onvoldoende tijd heeft om tot ontwikkeling te komen. Ook zou de tolerantie van deze planten toegeschreven kunnen worden aan de morfologie van het xyleem, dat nauwe vaten en weinig parenchym bevat, in tegenstelling tot de na infectie snel verwelkende planten van *Balsamina impatiens*, bij welke wijde vaten en veel parenchym in het xyleem voorkomt.

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