

# Evaluation of Italian Clones of Artichoke for Resistance to *Verticillium dahliae*

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## ABSTRACT

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Selected clones of artichokes were tested over 3 yr of perennial cropping to assess their resistance to *Verticillium* wilt. The disease severity was evaluated on the basis of external symptoms (ES) and vascular discoloration (VS). There were no artichoke clones which showed complete resistance, but based on disease incidence and severity indices, the clones could be grouped into susceptible, moderately resistant, and resistant classes. The most resistant clone, 76, had only 19.8 and 19.2% of plants showing VS and ES, respectively, and 9.2 and 10.3% severity indices for VS and ES, respectively. By comparison, clone 125, a susceptible check, had 100 and 97.5% of plants affected by VS and ES, respectively, with 79 and 78.7% severity indices for VS and ES, respectively. In general, the correlation coefficient between VS and ES was high. However, some resistant clones showed a disproportionately higher percentage of VS over ES, indicating that resistant clones may contain different forms of resistance, and that appraisal of both VS and ES is necessary to avoid over- or underestimating *Verticillium* resistance in artichoke germ plasm.

Additional keywords: *Cynara scolymus* L., partial resistance

Artichoke (*Cynara scolymus* L.) is grown in many countries in the world, but about 90% of the acreage is found in the Mediterranean area, with Italy being the largest producer (486,000 t, nearly 35% of world production). Spain and France rank second (444,000 t) and third (93,000 t), respectively. In the United States, artichoke is grown on the central coast of California, producing about 58,000 t (1,13). The first record of *Verticillium* wilt of artichoke was from Italy in 1928 (10,14). Four decades later, the disease was observed in Italy (7) and in southeastern France (3). During the 1980s, severe outbreaks of wilt, caused by *Verticillium dahliae* Kleb., were frequently observed in different artichoke-growing areas in Apulia (4,9), the largest Italian artichoke-growing district, producing 184,000 t (2). A decrease in artichoke acreage in some districts of Chile was also attributed to *Verticillium* wilt (11). Diseased plants show stunting, yellowing, wilting, and drying of the leaves, and punctiform or extensive vessel browning of the stem. Diseased plants do not yield, or produce a few smaller, deformed buds. Initially, the disease occurs on a few plants and subsequently spreads throughout the entire field (9). The strategy for controlling

*Verticillium* wilt of artichoke is based on planting in noninfested soil, the use of disease-free vegetatively produced plants, and long-term rotations with nonhost species (9). Soil fumigation with methyl bromide or dazomet provides nearly complete disease control (6), but this practice is limited by cost and by ecological constraints. The use of resistant cultivars would be more economical and ecologically sustainable. Recently, preliminary screening tests of 130 artichoke clones originating from several countries in the Mediterranean region showed a wide range of reactions to *Verticillium* wilt (5). In this paper, the results of field tests to assess some characteristics and the value of resistance of 11 selected clones of artichokes are presented.

## MATERIALS AND METHODS

Experiments were done in an open field near Bari in southern Italy. This field was artificially infested with *V. dahliae* and cropped with susceptible artichokes in the previous 6 yr. Eleven artichoke clones (72, 131, 106, 79, 71, 104, 113, 76, 75, 8, and 161) which in a preliminary test (5) showed different levels of resistance to the disease and three susceptible clones (125, 135, and 55) were tested. The experimental design consisted of three randomized blocks in which 120 plants of each clone were used. The plants were vegetatively obtained as rooted offshoots. Five isolates of *V. dahliae* from diseased artichokes growing in different locations of southern Italy were used. Inoculum was prepared by blending 10-day-old colonies grown on potato-saccharose agar in tap water. A final concentration of  $1 \times 10^6$  conidia

per milliliter was used. Inoculation was made by dipping the basal part of offshoots into the inoculum immediately before transplanting. Noninoculated plants of each artichoke clone were also grown in adjacent noninfested soil. The experimental field was maintained in perennial culture for three consecutive years, and the disease reaction of single plants was recorded. External disease symptoms were taken at monthly intervals during the vegetative phase of artichokes, and vascular browning was evaluated at the end of the second and third years of growth, when the plants were thinned out in June.

For each clone, the percentage of diseased plants and the disease severity index were estimated on the basis of external symptoms and vascular discoloration in the stem. The severity of external symptoms, including foliar yellowing and necrosis, and plant stunting, were assessed using a scale of six classes reported in Table 1. Quantitative evaluation of vascular discoloration was made on the main stem of each plant by cutting it transversely at soil level. The following scale for calculation of percent affected area was used: 0 = no visible discoloration, 1 = 1-9% stem discoloration, 2 = 10-24%, 3 = 25-50%, 4 = more than 50%, and 5 = dead plant. The formula used to calculate the disease severity indices (external symptoms or vascular discoloration) is  $I = \frac{\sum(f \times v)}{n \times x} \times 100$ , in which  $\Sigma$  = summation,  $f$  = frequency of a numerical rating,  $v$  = numerical rating,  $n$  = total number of tested plants, and  $x$  = maximum value of the scale.

Analysis of variance and Duncan's test were performed on severity disease indices and on percentages of diseased plants. CORR (15) and GPLOT (16) procedures of SAS System were used in the

**Table 1.** Rating scale (0-5) for estimating *Verticillium* wilt severity in individual artichoke plants based on leaf symptoms and stunting

Affected leaf surface (%)	Degree of stunting		
	None or very slight	Moderate	Severe
0	0	...	...
1-9	1	2	3
10-24	2	3	4
25-50	3	4	4
>50	4	4	4
Dead plants	5	5	5

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correlation analysis between external symptoms and vascular discoloration for both severity indices and percentage of diseased plants.

## RESULTS AND DISCUSSION

During all 3 yr of perennial culturing, clones of resistant artichokes exhibited percentage of diseased plants and disease severity indices which were statistically ( $P = 0.05$ ) lower than those for susceptible clones (Table 2). However, no artichoke clone showed complete resistance. At the end of the experiment, nearly 100% of plants of the susceptible clones 125, 135, and 55 had both external symptoms and vascular discoloration. Clone 76 was the most resistant, with the lowest percentage of plants affected by vascular discoloration (19.8%) and a low percentage with external symptoms (19.2%). The other clones of artichoke showed higher percentages of diseased plants, with vascular discoloration ranging from 43.3 to 75% (Table 2). In resistant artichokes, the percentage of plants affected by external symptoms ranged from 70.8 to 13.3%. At the end of the third year of testing, the vascular discoloration severity indices of susceptible clones ranged from 81.3 to 78.5%; while in resistant clones, indices remained below 45%. The indices for external symptom severity in the susceptible clones ranged from 80.7 to 78.7%, while those of resistant clones were below 40% (Table 2).

Based on the disease evaluation indices, the tested artichokes could be classified in three groups: resistant, including clones 8, 161, 76, 75, 113, and 104; moderately resistant, including clones 72, 131, 106, 79, and 71; and susceptible, including clones 125, 135, and 55 (Fig. 1). The correlation analysis between percentage of plants with external symptoms and percentage with vascular discoloration for all 14 clones yielded a correlation coefficient ( $r$ ) of

0.73 ( $P < 0.01$ ). The coefficient calculated for 11 clones (excluding clones 8, 104, and 161) was 0.93 ( $P < 0.01$ ). The latter three clones may possess resistance characters that are different from the other resistant clones. The correlation coefficient between severity of external symptoms and severity of vascular discoloration was 0.97 ( $P < 0.01$ ) (Fig. 1).

The development over time of wilt incidence and severity during the third year of cropping, i.e., from the last record taken in the second year to the final record in the third year, provided valuable evidence on resistance characteristics. During this period, the severity index and the incidence of external symptoms in clones with highest resistance remained nearly constant. For example, at the end of the second year of testing, the external symptoms in the resistant clone 113 produced a severity index of 11.4% and a disease incidence of 24.5%; at the end of the third year, the severity index and the incidence were 12.2 and 25%, respectively (Fig. 2). Moderately resistant and susceptible clones exhibited a progressive increase in external symptoms (severity and incidence). At the end of the second year, in clone 131, a moderately resistant clone, the severity index of external symptoms and the incidence were 9.8 and 22.5%, respectively; whereas at the end of the third year, the severity index and the disease incidence were 31.8 and 51.7%, respectively. During the same period, in clone 125, a susceptible clone, the severity index and the incidence of external symptoms increased from 38.6 to 78.7% and from 65.9 to 97.5%, respectively (Fig. 2).

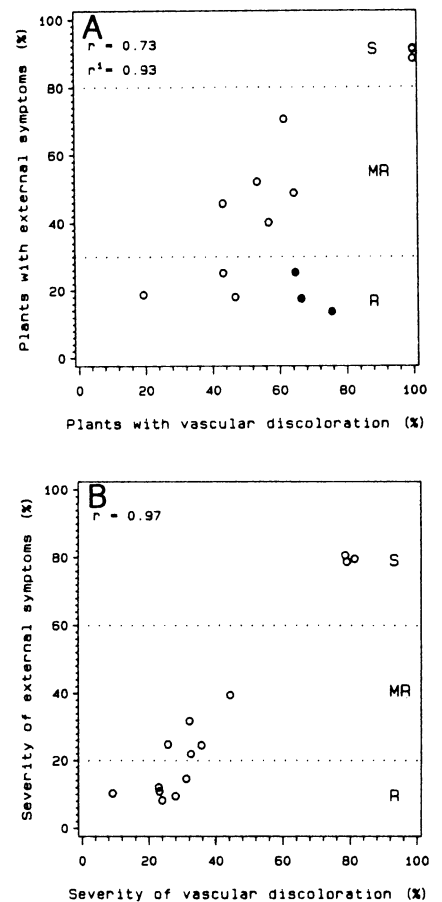
Intercrossing of resistant clones possessing diversified types of resistance should produce segregants with higher levels of resistance components expressing lesser external and internal disease symptoms. Commonly, *V. dahliae* causes external (flaccidity, wilting, chlorosis,

necrosis, and stunting) and internal (vascular discoloration, tyloses, gums) symptoms on host plants. However, in chrysanthemum (*Chrysanthemum morifolium* Ramat.) internal browning does not occur (12). It is very likely the two types of disease symptoms are expressions of different resistance components of the host. The occurrence of resistance based on a decrease of either or both external and internal symptoms has been described by Cirulli et al (8) in a screening study for *Verticillium* wilt resistance in eggplant. These authors found that more than 100 eggplant accessions could be classified, based on different combinations of external and internal disease components, into five resistance-susceptibility phenotypic groups. In the present study, artichoke clone 76 showed very low indices of both external and internal symptoms; whereas clones 8, 75, 104, 113, and 161 had very low external disease indices but moderate vascular discoloration (Table 2), thus indicating that appraisal for both external and internal disease expression is necessary in

**Table 2.** Field reactions of artichoke clones to *Verticillium* wilt at the end of 3 yr of perennial cropping

Artichoke clones	External symptoms		Vascular discoloration	
	Diseased plants (%)	Severity index	Diseased plants (%)	Severity index
125	97.5 a <sup>z</sup>	78.7 a	100 a	79.0 a
135	96.7 a	80.7 a	100 a	78.5 a
55	92.5 a	79.7 a	100 a	81.3 a
72	70.8 b	39.5 b	61.7 bc	44.2 b
131	51.7 c	31.8 bc	53.3 cd	32.0 cd
106	49.2 c	24.7 cd	64.2 bc	35.7 bc
79	45.8 c	24.8 cd	43.3 d	25.7 cd
71	40.0 cd	21.8 c-e	56.7 cd	32.3 cd
104	25.0 de	14.7 d-f	64.2 bc	31.2 cd
113	25.0 de	12.2 d-f	43.3 d	22.8 d
76	19.2 e	10.3 ef	19.8 e	9.2 e
75	18.3 e	10.8 ef	46.7 d	23.0 d
8	17.5 e	8.2 f	66.7 bc	23.8 d
161	13.3 e	9.3 ef	75.0 b	27.8 cd

<sup>z</sup> Means in columns followed by the same letter do not differ significantly at  $P = 0.05$ , according to Duncan's multiple range test.



**Fig. 1.** Relationship between (A) incidence and (B) severity of external and internal symptoms in susceptible, moderately resistant, and resistant clones of artichokes. S = susceptible clones 55, 125, and 135; MR = moderately resistant clones 71, 72, 79, 106, and 131; R = resistant clones 8, 75, 76, 104, 113, and 161;  $r$  = correlation coefficient for all 14 clones; and  $r^2$  = correlation coefficient for all clones excluding clones 8, 104, and 161 (●).

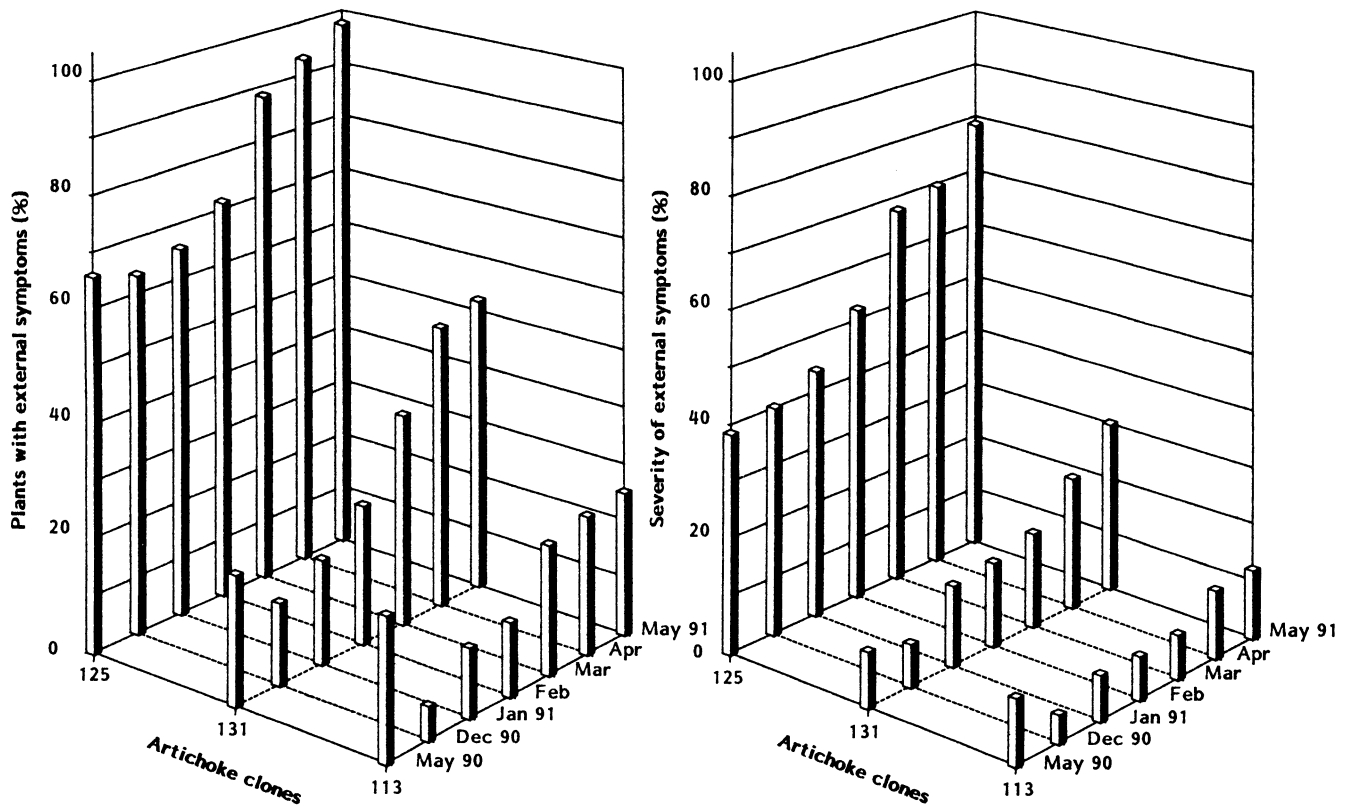


Fig. 2. The development over time of *Verticillium* wilt in clones 125, 131, and 113, representing susceptible, moderately resistant, and resistant artichokes, respectively (during the third year of perennial cropping).

the search of artichoke germ plasm with resistance to *V. dahliae*. The offshoot inoculation combined with growing the plants in field soil infested by *V. dahliae* provided an accurate method of screening artichokes for resistance to *Verticillium*. This method, although requiring time and space, is superior to greenhouse screening for two reasons: over the 3 yr of perennial growing, the possibility of detecting small differences in resistance is greater; and in the greenhouse, artichoke plants grow poorly and wilting symptoms can be confused by early senescence of basal leaves (*unpublished observations*).

Resistant artichoke clones 8, 76, 104, 113, and 161 are being commercially grown in specific artichoke districts of Italy and possess selected horticultural characters and climatic adaptation. Thus, they constitute a valuable germ plasm in artichoke breeding for *Verticillium* resistance and general cultivar improvement.

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