SUMMARY

Dills (Anethum graveolens L.) grown from seeds in a small area of the Italian Riviera showed symptoms including stalk discoloration, flower virescence and phylody. The affected plants were tested for the presence of phytoplasmas. DNAs were PCR-amplified in reactions with ribosomal universal or group-specific primer pairs and analysed by restriction digestions with BfaI or MseI enzyme. Phytoplasmas, identified as members of 16SrI-B, I-C, V-A and XII-A subgroups were present in 18 out of 22 samples (81.8%). Of these, 9 (50% of the infected) carried a single phytoplasma [3 subgroup 16SrI-B (16.6%), 1 16SrI-C (5.5%), and 5 16SrV-A (27.7%)]. Seven samples (38.8%) carried two different phytoplasmas (6 members of subgroups V-A and XII-A; 1 member of subgroups I-C and V-A). Two samples carried phytoplasmas of three different subgroups in different mixes (I-C, V-A and XII-A; I-B, I-C and XII-A). There was no clear correlation between a given symptom and the presence of one or more phytoplasmas. This is the first report from Europe of phytoplasmas in dill plants.

Key words: dill, phytoplasma, PCR detection, RFLP analysis.

Within the class Mollicutes, the monophyletic clade of the phytoplasmas includes 14 16S rDNA RFLP groups, subdivided into 32 subgroups (Lee et al., 1998), that encompass the phytoplasmas associated with hundreds of diseases distributed worldwide in several plant species (Lee et al., 2000). Symptomatic plants may exhibit generalised stunting, unseasonal yellowing or reddening, abnormal proliferation of axillary buds resulting in witches'-broom appearance, decline and dieback, flower virescence and/or phyllody. Different phytoplasmas may cause identical symptoms in certain plant species, whereas closely related ones can induce different syndromes in the same host (Lee et al., 2000).

In the agronomically important region of the Italian Riviera, a number of plant diseases, involving ornamental and horticultural crops, wild shrubs and cultivated trees, have been associated with several phytoplasmas, mainly of the subgroups I-B and XII-A, and less frequently I-C (Bertaccini et al., 1990, 1992; Minucci et al., 1994, 1995; Vibio et al., 1994, 1995; Conti et al., 1997; Marzachi et al., 1999, 2000; Alma et al., 2000). Representatives of subgroups V-C and V-E have been identified from diseased grapevines and stunted blackberry, respectively (Bertaccini et al., 1995; Lee et al., 1995b; Osti et al., 2000; Davis and Dally, 2001).

The cultivation of aromatic dill (Anethum graveolens L.), grown from seeds in open fields in Spring and Autumn or, during Winter, under protected conditions, has been recently introduced to the region and maintained mainly for export. During March-April 2000 and 2001 we have observed, in both types of cultivation, the appearance of stunted plants showing whitening or reddening of the stalks, flower virescence, phyllody and witches'-broom, with almost complete loss of production. Over time, the incidence of such syndrome has considerably increased, affecting over 20% of the crops.

DNAs were extracted as described (Marzachi et al., 1999) and amplified in assays driven by the universal primers FP1/rP7 (Schneider et al., 1995). Irrespective of the results of direct amplifications, reaction mixtures were diluted 1:40 and subjected to nested PCRs separately by universal primers R16F2/R2 (Lee et al., 1993) and one of the following group-specific primer pairs: R16(I)F1/R1, R16(III)F2/R1, R16(V)F1/R1 (Lee et al., 1994) or R16(X)F1/R1 (Lee et al., 1995a), according to conditions detailed in the original papers. Products were analysed in 1% (w/v) agarose gels and visualised by UV light as described (Marzachi et al., 1999). Amplified fragments were restricted with appropriate enzymes following manufacturer’s instructions and fractionated in 5% (w/v) polyacrylamide gels as described (Marzachi et al., 1999). Phytoplasma reference isolates [acronyms and subgroup of affiliation (sensu Lee et al., 1999)].

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1998) in parenthesis], maintained and propagated in penkwinkles \textit{(Catharanthus roseus (L.) G. Don)}, were: American aster yellows (AAY; I-A), European aster yellows (EAY; I-B), clover phyllody (CPh; I-C), peach yellow leaf roll (III-A), Vaccinium witches’ broom (III-B), American elm yellows (EY-1; V-A), Serbian stolbur strain from pepper (Stol; XII-A). DNAs from apple proliferation and pear decline phytoplasmas (X-A and X-C, respectively), jujube (\textit{Zizyphus jujuba} Miller), blackberry (\textit{Rubus fruticosus} L.) and grapevine (\textit{Vitis vinifera} L.), naturally infected with jujube witches’ broom (JWB; V-C) and Flavescence dorée (FD; V-C) phytoplasmas, respectively (Pasquini et al., 2000), were also used.

No correlation between a distinctive symptom and a given phytoplasma could be determined from the results of PCR and RFLP analyses of samples listed in Table 1. Eighteen out of 22 samples (81.8%) did respond positively in nested assays with universal and groups I- and V-specific primer pairs. Failure to amplify phytoplasma DNA in 9% of the symptomatic samples could have been due to titres below detection limits or to the reduced size (1.5 g of leaf veins) of the analysed samples. Nine samples (50%) yielded phytoplasma-specific amplicons with both these primer pairs, thus indicating multiple infections with phytoplasmas of different groups (Table 1). Only the reference isolates, not the samples, yielded the expected phytoplasma-specific fragments using groups 16SrIII- or X-specific primer pairs (not shown).

\textit{BfaI}-digested amplicons obtained from dill DNA by nested PCR using group V-specific primer pair appeared indistinguishable among them and from those of V-A or V-B subgroups reference isolates, but differed from those of subgroup V-E (examples in Fig. 1). As subgroup V-B members have been reported only in China from jujube (\textit{Z. jujuba}) and Chinese cherry (\textit{Prunus pseudocerasus} Lindl.) (Zhu et al., 1998), it is conceivable that these phytoplasmas belonged to subgroup V-A. Group V phytoplasmas have been detected in diseased tomatoes (\textit{Lycopersicum esculentum} Mill) from central Italy (Del Serrone et al., 2001) and \textit{Celtis australis} L. (Bertaccini et al., 1996). In Italy group V-A phytoplasmas have been detected in \textit{Ulmus} spp. (Griffiths et al., 1999) and \textit{Eucaliptus} spp. (Camele et al., 1999).

\textit{MseI}-RFLP profiles of 16S rDNA sequences amplified by nested PCR with group I-specific primer pair indicated that the majority of the positive samples were carrying phytoplasmas indistinguishable from the reference stolbur isolate (XII-A, Fig. 2 S and samples 9 and 6), whereas samples 3, 16 and 17 (Table 1) yielded restriction patterns identical to that of AAY and EAY phytoplasma (I-A and I-B, Fig. 2, A and E, respectively) and sample 21 had the same appearance as CPh phytoplasma (I-C, Fig. 2C).

However, subgroup I-A members appear to be restricted to North America (Seemüller et al., 1998; Lee et al., 1998, 2000), and therefore it is likely that

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nested (universal)</th>
<th>Nested (I)</th>
<th>Nested (V)</th>
<th>RFLP</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>2</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>V-A</td>
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<tr>
<td>3</td>
<td>+</td>
<td>+</td>
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<td>I-B</td>
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<td>6</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>V-A, XII-A</td>
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<td>–</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
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<td>V-A</td>
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<td>+</td>
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<td>–</td>
<td>I-B</td>
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<td>+</td>
<td>+</td>
<td>–</td>
<td>I-B</td>
</tr>
<tr>
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<td>+</td>
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<td>–</td>
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<tr>
<td>19</td>
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<td>–</td>
<td>+</td>
<td>V-A</td>
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<tr>
<td>20</td>
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<td>–</td>
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<td>–</td>
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<tr>
<td>21</td>
<td>+</td>
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</table>
infected dills were in fact harbouring subgroup I-B phytoplasmas. Multiple infection by members of different subgroups was suggested by the complex RFLP patterns of samples 10 and 18 (Fig. 2). As mentioned, in the Italian Riviera, group I phytoplasmas appear to be the commonest in various crops and wild plants (Bertaccini et al., 1990, 1992; Conti et al., 1997; Marzachì et al., 1999). This may well be due to the fact that they are efficiently vectored by several species of polyphagous leafhoppers (Guglielmone et al., 1994; Bosco et al., 1997; Alma et al., 2000) and hence it is no surprise that new crops may become their targets.

Samples 6 and 9 showed identical RFLP patterns, indistinguishable from that of the reference stolbur isolate (Fig. 2), in line with previous reports (Bertaccini et al., 1995; Marzachì et al., 1999, 2000). While different stolbur isolates have been described from field bindweed (Convolvulus arvensis L.) from southern Italy and south western Germany (Schneider et al., 1997). Two cicid hoppers (Hyalesthes obsoletus Signoret and Pentastiridius beieri Wagner) had been demonstrated as vectors of stolbur (Fos et al., 1992; Gatineau et al., 2001). However, stolbur appears to actively spread within vineyards in the absence of H. obsoletus in Spain (Batlle et al., 2000).

Yield losses in dill crops have been in the past attributed mainly to fungal attacks (Fusarium sp.; Frauenstein, 1968), but also, in the former German Democratic Republic and Italy, to sporadic alfamo-, cucumo- and poty-virus infections (Wolf, 1972; Bellardi et al., 1998). An outbreak of aster yellows phytoplasmas (I-A and I-B) in dills, among other vegetable crops and biennial weeds, has recently been reported from south western Texas (Lee et al., 2001).

We here report that dills can be affected, in single, double or triple infection, by phytoplasmas belonging to subgroups I-B, I-C, V-A and XII-A. Although one or more phytoplasmas could not be linked to any given syndrome, they appear to be rather common in the area surveyed and frequently associated with the dill crop. The increasing number of symptomatic plants over successive years indicates that dills, as newly introduced crop, are susceptible to phytoplasma infection.

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