The Distinctive Morphology of ‘*Fragaria multiplicata*’ Is Due to Phytoplasma

Rasa Jomantiene¹, Robert E. Davis^{1,3}, Ellen L. Dally¹, and John L. Maas²
U.S. Department of Agriculture, Agricultural Research Service, Beltsville,
MD 20705

Joseph D. Postman

National Clonal Germplasm Repository, U.S. Department of Agriculture,
Agricultural Research Service, Corvallis, OR 97333

Additional index words. nested-PCR, mycoplasma-like organism, strawberry, multiplier disease, strawberry germplasm

Abstract. The rare plant, *Fragaria multiplicata* Fern., was characterized by an unusual vegetative morphology that was long presumed to be suggestive of an ice front relict. While an additional species of *Fragaria* would be a potential source of genetic diversity for enhancing cultivated strawberry germplasm, evidence now indicates that such potential is not present in *F. multiplicata*. Grafting of *F. multiplicata* to *F. chiloensis* Duchesne resulted in transmission of a subgroup 16SrVI-B phytoplasma to, and the development of multicapital growth in, *F. chiloensis*. The results indicated that *F. multiplicata* is a phytoplasma-diseased aberrant growth form of *F. virginiana* Duchesne and is an unfounded taxon. It is apparent that this plant population offers no unique potential for increasing genetic diversity in cultivated strawberry germplasm, but the phytoplasma may be capable of infecting commercial strawberry.

Fragaria multiplicata Fern. was described as a new species of strawberry by Fernald (1908) from populations collected in 1906 from isolated sites along the Rivière Ste.-Anne in Gaspé, Que., on the Gulf of St. Lawrence. Plants of this rare species were characterized as being dwarfed, with short petioles, small leaves and flowers, and a multi-branched crown that often developed 30 to 40 branch crowns (Fernald, 1908). The vegetative morphology of *F. multiplicata* was suggestive of a subarctic or ice front relict (Catling, 1993; Catling et al., 1995). Plants of *F. multiplicata* were not noted again, and the species was thought to be extinct, until its rediscovery on the Gaspé Peninsula in 1992 (Catling, 1993). Existence of the species implied that *F. multiplicata* could represent genetic diversity of potential significance in broadening the germplasm base of North American cultivated strawberries (Catling, 1993; Catling et al., 1995).

The morphotype of *F. multiplicata* also resembles that of commercial strawberry (*Fragaria ×ananassa* Duch.) affected by the multiplier disease found in the Great Lakes

region of the United States and Canada and in British Columbia, Canada (Converse, 1987; Heimann et al., 1992; Maas, 1984). Some have speculated that the distinctive growth habit of *F. multiplicata* may be due to disease (Reed, 1966). Staudt in 1960 annotated the type specimen of *F. multiplicata* (Fernald & Collins, no. 230, Gray Herbarium, Harvard Univ., in Catling, 1993) as representing a variant of *Fragaria virginiana* Duch. ssp. *glauca* (S.Wats.) Staudt, a common North American strawberry, and noted that the *F. multiplicata* taxon probably is invalid (Staudt, personal communication). Catling et al. (1995) transmitted a putative pathogen from typical *F. multiplicata* plants to previously healthy plants of *F. chiloensis* Duchesne, which then developed symptoms of multiplier disease. However, in the same study they proposed to retain taxonomic recognition of *F. multiplicata*, reducing it to the rank of forma: *Fragaria virginiana* ssp. *glauca* f. *multiplicata* (Fern.) Catling & J. Cayouette.

We undertook to investigate whether phytoplasma may be responsible for the unusual vegetative morphology of *F. multiplicata* for several reasons: The vegetative morphology resembled the growth form of cultivated strawberry plants affected by multiplier disease, healthy plants of *F. chiloensis* grafted with tissues from *F. multiplicata* subsequently developed symptoms of multiplier disease (Catling et al., 1995; Jomantiene et al., 1997), and phytoplasmal infection has been associated with multiplier disease in cultivated strawberry (Harrison et al., 1997; Heimann et al., 1992). We report the results of our study establishing that the vegetative morphology of *F. multiplicata* is indeed due to infection by a previously unrecognized, putative phytoplasma species.

Plant samples and reference phytoplasma strains. Healthy *F. chiloensis* plants were grafted with tissue from *F. multiplicata* by the leaf insertion method (Maas, 1984) and vegetatively propagated in a greenhouse as clone FRA9083. Samples of leaves were collected from two plant clones (FRA1370 and FRA1371) of *F. multiplicata*, the grafted clone (FRA9083), and four healthy, nongrafted clones (FRA-0711, CA-11, FRA-0808, and Del Norte) of *F. chiloensis* in the National Plant Germplasm Collection in Corvallis, Ore. Clover proliferation (CP) phytoplasma, originally kindly supplied by C. Hiruki (Univ. of Alberta, Edmonton, Alta., Canada), and aster yellows (AY) phytoplasma (Lee et al., 1993) were maintained in a greenhouse in grafted plants of a white flowered clone of periwinkle [*Catharanthus roseus* (L.) G. Don] and used as positive control in the polymerase chain reaction (PCR) or as reference in restriction fragment length polymorphism (RFLP) analyses of phytoplasma DNAs.

Polymerase chain reaction conditions and primers. Nucleic acid for use as template in the PCR was extracted from fresh leaf tissue by a method previously described (Lee et al., 1993). After extraction, DNA was purified using GeneCleanIII Kit as specified by the manufacturer (BIO 101, Inc., Vista, Calif.).

Two pairs of oligonucleotides, phytoplasma universal primer pairs R16mF2/R1 and R16F2n/R2 (Gundersen and Lee, 1996), were used to prime amplification of phytoplasma 16S rDNA (16S rRNA gene) sequences in nested PCRs. In the nested PCRs, DNA amplified in PCR primed by R16mF2/R1 was diluted 1:50 with sterile distilled water and used as template in PCR primed by R16F2n/R2. Each PCR was carried out in a final volume of 25 µL of reaction mixture as previously described (Gundersen and Lee, 1996). DNA fragment size standard was 1 kb ladder (Life Technologies, Gaithersburg, Md.). PCRs were carried out for 35 cycles using the following parameters: 1 min (2 min for first cycle) denaturation at 94 °C, annealing for 2 min at 55 °C, and primer extension for 3 min (10 min in final cycle) at 72 °C. Reaction mixtures devoid of templates were included in all experiments as negative controls. PCR products were analyzed by electrophoresis through 1% agarose gel, stained with ethidium bromide, and DNA bands visualized using a UV transilluminator.

RFLP analyses of amplified phytoplasma DNA. Products from nested PCR primed by R16F2n/R2 were analyzed by single enzyme digestion, according to endonuclease manufacturers' instructions, with *AluI*, *HhaI*, *KpnI* (Life Technologies), and *DraI*, *HaeIII*, *HinfI*, *HpaI*, *HpaII*, *MseI*, *RsaI*, and *Sau3AI* (New England Biolabs, Beverly, Mass.). The restriction fragment length polymorphism (RFLP) profile of digested DNA was analyzed by electrophoresis through 5% polyacrylamide gel, staining in ethidium bromide, and visualization using a UV transilluminator. DNA fragment size standards used were PhiX174 RF *HaeIII* digest (Life Technolo-

Received for publication 12 Feb. 1998. Accepted for publication 27 Apr. 1998. We thank Kristi Bottner for excellent technical assistance, P.M. Catling for kindly supplying live samples of *F. multiplicata*, and A.Y. Rossman and J.H. Wiersma for critical reading of the manuscript. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

¹Molecular Plant Pathology Laboratory.

²Fruit Laboratory.

³To whom reprint requests should be addressed. e-mail: rdavis@asrr.arsusda.gov

gies) and 1.6 kb ladder (MBI Fermentas, Vilnius, Lithuania). The RFLP patterns were compared with those obtained using reference phytoplasmas in this study and with RFLP patterns previously published (Davis et al., 1997a; Lee et al., 1993; Vibio et al., 1994).

Nucleotide sequencing and putative restriction site analysis. The PCR-amplified 16S rRNA gene products were sequenced using standard dideoxy termination methods and automated DNA sequencing. The nucleotide sequences determined in this study were deposited in the GenBank (National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, Md.) data library. A partial sequence of clover proliferation (CP) phytoplasma 16S rDNA (GenBank accession no. L33761) was obtained from GenBank. In the present work, we amplified and sequenced 16S rDNA from CP phytoplasma in order to verify the GenBank 16S rDNA sequence from CP phytoplasma and to complete nucleotide sequencing of 43 bases missing from GenBank accession no. L33761. Gene sequences from CP phytoplasma and from the phytoplasma detected in *F. multicipita* in this study were analyzed to estimate sequence similarities and to identify recognition sequences for selected restriction enzymes. Putative restriction site maps were generated by using the DNASTAR program MapDraw option (DNASTAR, Madison, Wis.). Alignment of pairs of sequences were generated and sequence similarities evaluated by using the DNASTAR program Align option.

Results

Detection of a phytoplasma in *F. multicipita* and grafted *F. chiloensis*. When healthy *F. chiloensis* plants were grafted with tissue from

F. multicipita, they produced small leaves, multiple crowns, and no runners (Fig. 1). Vegetatively propagated progeny from the grafted plants exhibited the same symptoms. Since the growth form of *F. multicipita* and the symptoms in grafted *F. chiloensis* plants were suggestive of phytoplasma infection, we assayed plants of *F. multicipita* and of grafted *F. chiloensis* for presence of phytoplasma by using the polymerase chain reaction (PCR) in attempts to amplify phytoplasma 16S rDNA from such plants. No DNA amplification was observed in PCR containing template DNA from healthy, nongrafted *F. chiloensis* plants. In contrast, amplification of phytoplasma-specific 1.2 kbp DNA sequences was observed in all nested PCRs that contained template DNAs derived from *F. multicipita* or from grafted *F. chiloensis*, indicating infection of these plants by phytoplasma (Fig. 2).

RFLP analysis of PCR-amplified phytoplasma 16S rDNA. Initial assignment of the detected phytoplasmas to groups and subgroups was accomplished by RFLP analysis of amplified 16S rDNA from reactions primed by primer pair R16F2n/R2. The phytoplasma strains detected in the *F. multicipita* and the grafted *F. chiloensis* plants were similar or identical to one another in collective RFLP patterns of 16S rDNA analyzed by use of *AluI*, *MseI*, *KpnI*, *DraI*, *HinfI*, *HpaI*, *HpaII*, *HhaI*, *HaeIII*, *RsaI*, and *Sau3AI*. On the basis of these results, we tentatively interpreted the phytoplasma strains as belonging to a single putative species. Two of the strains, one from *F. multicipita* and one from grafted *F. chiloensis*, were chosen for further work. Comparison of the RFLP patterns of 16S rDNA from the *F. multicipita* phytoplasma strains with patterns previously published for 16S rDNA from other phytoplasmas revealed that

the *F. multicipita* phytoplasma was affiliated with group 16SrVI [clover proliferation (CP) and related phytoplasmas] (Fig. 3). Since the *F. multicipita* phytoplasma was distinguished from CP phytoplasma by RFLP analysis of 16S rDNA with *AluI* (Fig. 3), we established two new subgroups to encompass these phytoplasmas, subgroup 16SrVI-A (type strain, CP phytoplasma) and subgroup 16SrVI-B (type strain, the *F. multicipita* phytoplasma).

Nucleotide sequences and putative restriction sites in phytoplasma 16S rRNA gene sequences. Since the amplified DNAs from both *F. multicipita* plants and from the grafted *F. chiloensis* plant were mutually indistinguishable by the RFLP analyses, one phytoplasma (that in grafted *F. chiloensis* plant FRA9083) was termed strain FRA9083 and chosen for further characterization. The nucleotide sequence was determined for an ≈1.2 Kbp 16S rDNA amplified in a PCR containing primer pair R16F2n/R2 and template DNA derived from the grafted *F. chiloensis* plant FRA9083. The sequence has been deposited in GenBank (GenBank Accession No. AF036354). Since RFLP analysis of amplified 16S rDNA revealed that the *F. multicipita* phytoplasma was a member of group 16SrVI (clover proliferation and related phytoplasmas), we also determined the sequence for a missing 43-base segment of a previously published 16S rDNA sequence (GenBank Accession No. L33761) from clover proliferation (CP) phytoplasma (type strain of group 16SrVI), and compared the *F. multicipita* and CP phytoplasma 16S rDNA sequences. The sequence of the 43-bases determined for CP phytoplasma 16S rDNA was identical to the corresponding base

Fig. 1. Multicipitate vegetative morphology induced in *Fragaria chiloensis* grafted with tissue from *F. multicipita*. (Left) Grafted *F. chiloensis* carrying tissue from *F. multicipita*. (Right) Normal, ungrafted plant of *F. chiloensis*. The grafted plant displays multiple crowns, reduced size of leaves, and lack of runners.

Fig. 2. Nested polymerase chain reaction (PCR) amplification of phytoplasma 16S rDNA from field collected plants of *F. multicipita* and grafted *F. chiloensis*. The first PCR was primed with primer pair R16mF2/R1, and this was followed by reamplification of target DNA in a nested PCR primed with primer pair R16F2n/R2. Lane S: 1 kbp size standard; lane W: products of PCR that was devoid of template DNA; lanes AY, 1370, 1371, G, H1, H2, H3, H4: template DNAs derived from a periwinkle plant affected by aster yellows disease (AY), a field-collected *F. multicipita* clone FRA-1370 (1370), a field-collected *F. multicipita* clone FRA-1371 (1371), a grafted *F. chiloensis* clone 9083 (G), and healthy, nongrafted *F. chiloensis* clones FRA-0711 (H1), CA-11 (H2), F00808 (H3), and Del Norte (H4), respectively.

Fig. 3. RFLP analysis of 16S rDNAs from phytoplasma strains detected in *F. multicipita* and grafted *F. chiloensis* by use of nested PCR. DNA products from the nested PCR, primed by R16F2n/R16R2, were digested with restriction endonucleases (left) *AluI*, *MseI*, *KpnI*, *HpaII*, *HpaI* and (right) *HhaI*, *HaeIII*, *RsaI*, *Sau3AI*, *DraI*, *HinfI*. Lane S1 contained PhiX174 RFI DNA *HaeIII* digest; the fragment sizes in this lane were (from top to bottom) 1353, 1078, 873, 603, 310, 281, 271, 234, 194, 118, and 72 bp. Lane S2: 1.6 kbp ladder size standard. Lanes CP, 1370, and G: digests of DNAs amplified from clover proliferation phytoplasma (CP) and from phytoplasmas detected in field collected *F. multicipita* clone FRA-1370 (1370) and in grafted *F. chiloensis* clone 9083 (G).

positions of 16S rDNA from the *F. multicipita* phytoplasma strain FRA9083 (data not shown). The *F. multicipita* and CP 1.2 Kbp 16S rDNA sequences shared $\approx 98.5\%$ sequence similarity. Results from comparative analysis of putative restriction sites in the sequenced DNAs are shown in Fig. 4. Expected fragment sizes based on analysis of putative restriction sites were in excellent agreement with fragment sizes obtained by enzymatic RFLP analysis of amplified 16S rDNA (Fig. 3). The *F. multicipita* phytoplasma and CP 16S rDNAs differed by

the presence in CP phytoplasma 16S rDNA of an *AluI* site (see arrow in Fig. 4) that was absent from the 16S rDNA of the *F. multicipita* phytoplasma.

Discussion

Our study of an extant population of *F. multicipita* has revealed that the unusual vegetative morphology that is characteristic of this taxon coincided with infection of the plants by a phytoplasma. As previously noted

(Catling et al., 1995), the vegetative morphology of *F. multicipita* is typical of strawberry plants affected by multiplier disease, which has been attributed to phytoplasma infection (Heimann et al., 1992). Coincidence of the multicipitate morphotype and phytoplasma infection in *F. multicipita* (this paper) is consistent with the hypothesis that the morphotype is induced by a plant pathogenic phytoplasma. This view is supported by the reversion, following antibiotic treatments and heat therapy, of subclones of *F. multicipita* to a morphotype

Fig. 4. Analyses of putative restriction sites in phytoplasma 16S rRNA gene sequences. Maps were generated by using the MapDraw option of the DNASTAR program (DNASTAR, Madison, Wis.) and were manually aligned for comparison of recognition sites for restriction endonucleases *AluI*, *DraI*, *HaeIII*, *HhaI*, *HinfI*, *HpaI*, *HpaII*, *MseI*, *RsaI*, and *TaqI*. Arrow indicates the presence in CP phytoplasma 16S rDNA of an *AluI* site that was absent in the 16S rDNA of the *F. multicipita* phytoplasma.

that is characteristic of normal *F. virginiana* (Postman et al., 1997). Induction of the *F. multicipita* vegetative morphology by a phytoplasma is also indicated by the finding that graft transmission of a multicipitate growth form-inducing agent from *F. multicipita* to normal *F. chiloensis* coincided with transmission of a specific phytoplasma (this paper). Together, these results support the conclusion that *F. multicipita* is a unique growth form induced in a *Fragaria* sp. by phytoplasmal infection.

Although floral morphology has played a dominant role in the taxonomy of vascular plants, characteristics of vegetative morphology have played significant roles at the species level and lower taxonomic ranks (Stuessy, 1990). Indeed, arguments have been put forth for maintaining and increasing the use of vegetative morphology in plant taxonomy, in part because of the adaptive value of vegetative morphology and its significance for endemism (Tomlinson, 1984). However, some pathogens, including phytoplasmas, that systemically infect their plant hosts can induce morphological variants of which the growth habits are stable and distinctly different from those of various related taxonomic entities. Plants infected by phytoplasmas typically exhibit growth habits suggestive of significant alterations in the normal balance of plant growth hormones (Davis and Lee, 1982; Lee and Davis, 1992). Changes in vegetative morphology induced by phytoplasmal infections include dwarfing, development of abnormally small leaves, formation of simple leaves rather than normal compound leaves, deliquescent branching, reduced root growth, abnormal proliferation of axillary shoots, and development of multiple crowns. The actual array of changes in vegetative morphology varies with the specific phytoplasma pathogen-plant host interaction. Some changes in morphology have even produced horticulturally desirable plant traits, such as the free-branching growth habit in commercial poinsettia (Lee et al., 1997), but most have had well-recognized deleterious effects on the growth of wild or cultivated plants (Davis and Lee, 1982; Lee and Davis, 1992). Evidence now available (this paper and Catling et al., 1995) indicates that an unusual vegetative morphology induced by phytoplasmal disease in an isolated population of a wild, native *Fragaria* prompted the taxonomic description of a rare taxon, *Fragaria multicipita*.

As in our work, Catling et al. (1995) also found that the growth habit of *F. multicipita* could be transmitted by grafting to normal *F. chiloensis*, which then developed a growth form that resembled the symptom syndrome of strawberry multiplier disease. While noting that their observations suggested "that *F. multicipita* is only a diseased form of *F. virginiana*...", these authors also noted "that the advantage in retaining a rank for the name multicipita is that it enables the classification system to account for the unusual morphology, which would otherwise continue to raise questions not readily answerable by a systematist." Thus, *F. multicipita* was retained as a

taxonomic entity, the species being reduced to the taxonomic rank of forma as a new combination, *Fragaria virginiana* ssp. *glauca* f. *multicipita* (Fern.) Catling & J. Cayouette (Catling et al., 1995). In contrast to this view of taxonomic rank, there are numerous examples of phytoplasma-diseased plants having unusual growth forms (Lee and Davis, 1992; Lee et al., 1997). In phytoplasma-infected strawberry plants, such morphotypes are generally considered simply as diseased forms of *Fragaria*, rather than as distinct taxonomic entities (Maas, 1984). Although our results lead us to the same conclusion as that of Catling et al. (1995), namely, that *F. multicipita* is putatively a diseased form of *F. virginiana*, we further conclude that *F. multicipita* is an erroneous taxon without genotypic basis. This is the first reported example of a plant taxon invalidated due to the finding that its distinctive morphotype is induced by a phytoplasma pathogen.

While the available evidence (this paper; Catling et al., 1995; Fernald, 1908; Jomantiene et al., 1997) points to *F. multicipita* as a natural population of phytoplasma-infected *F. virginiana*, it also prompts further recognition of what has been noted by Luby et al. (1992) as a relatively narrow germplasm base underlying North American strawberry production. Catling (1993) suggested that natural variation in native wild strawberries, including *F. multicipita*, has relevance to agriculture and warrants in situ protection of crop-relative plant diversity such as that observed in the valley of Riviere Ste.-Anne. However, the current disposition of *F. multicipita* indicates that this plant population offers no potential for enhancement of the North American germplasm base that is available for increasing the genetic diversity of cultivated strawberries.

Whereas the *F. multicipita* phytoplasma has evidently occurred naturally in isolated populations of *F. virginiana* for nearly a century (this paper and Jomantiene et al., 1997), it is significant that this pathogen also infects *F. chiloensis*, at least experimentally by graft inoculation. Presumably then, this pathogen could also infect the commercial strawberry, *Fragaria xananassa*, which originated as an interspecific hybrid between *F. virginiana* and *F. chiloensis*. While the *F. multicipita* phytoplasma belongs to 16S rRNA gene group 16SrVI (clover proliferation, potato witches broom, and related phytoplasmas) (sensu Lee et al., 1993), it probably represents a distinct gene pool. We postulate that the 16S rDNA nucleotide substitutions observed between the *F. multicipita* and CP phytoplasmas signal divergence of the *F. multicipita* phytoplasma from other group 16SrVI phytoplasmas. The distinctive 16S rRNA gene sequence of this phytoplasma will make it feasible to trace its occurrence in potential plant hosts in various geographic regions. Recent evidence indicates a possible link between the *F. multicipita* phytoplasma and a phytoplasma associated with a disease problem currently affecting commercial strawberry plantings in Florida (Davis et al., 1997b).

Literature Cited

- Catling, P.M. 1993. Rediscovery of the many-crowned strawberry, *Fragaria multicipita* Fernald. *Rhodora* 95:225-233.
- Catling, P.M., J. Cayouette, and J. Postman. 1995. *Fragaria multicipita*, reduced to the rank of forma. *Rhodora* 97:245-254.
- Converse, R.H. 1987. Strawberry witches'-broom and multiplier diseases, p. 66-68. In: R.H. Converse (ed.). *Virus diseases of small fruits*. U.S. Dept. Agr. Hbk. 631. Washington, D.C.
- Davis, R.E., E.L. Dally, D.E. Gundersen, I.-M. Lee, and N. Habili. 1997a. "Candidatus Phytoplasma australiense," a new phytoplasma taxon associated with Australian grapevine yellows. *Intl. J. System. Bacteriol.* 47:262-269.
- Davis, R.E., R. Jomantiene, E.L. Dally, J. Maas, D. Legard, and J. Postman. 1997b. Possible link between the rare plant taxon "*Fragaria multicipita*" in Canada and a disease problem in strawberry in Florida. *Acta Hort.* 471: (In press.)
- Davis, R.E. and I.-M. Lee. 1982. Pathogenicity of spiroplasmas, mycoplasma-like organisms, and vascular-limited fastidious walled bacteria, p. 491-513. In: E. Civerolo, A. Collmer, R.E. Davis, and A.G. Gillaspie (eds.). *Phytopathogenic prokaryotes*. vol. I. Academic, New York.
- Fernald, M.L. 1908. Notes on some plants of northeastern North America. *Rhodora* 10:46-55.
- Gundersen, D.E. and I.-M. Lee. 1996. Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. *Phytopath.* 86:144-151.
- Harrison, N.A., D.E. Legard, R. DiBono, and P.A. Richardson. 1997. Detection and differentiation of phytoplasmas associated with diseases of strawberry in Florida. *Plant Dis.* 81:230.
- Heimann, M.F., R.N. Spear, and S.N. Jeffers. 1992. Mycoplasma-like organisms associated with strawberry multiplier disease. *Phytopathology* 82:1133.
- Jomantiene, R., R.E. Davis, E.L. Dally, J. Maas, and J. Postman. 1997. Evidence that "*Fragaria multicipita*" is a phytoplasma-diseased *Fragaria*. *Phytopathology* 87:549. (Abstr.)
- Lee, I.-M. and R.E. Davis. 1992. Mycoplasmas which infect plants and insects, p. 379-390. In: J. Maniloff, R.N. McElhane, L.R. Finch, and J.B. Baseman (eds.). *Mycoplasmas: Molecular biology and pathogenesis*. Amer. Soc. Microbiol., Washington, D.C.
- Lee, I.-M., R.W. Hammond, R.E. Davis, and D.E. Gundersen. 1993. Universal amplification and analysis of pathogen 16S rDNA for classification and identification of mycoplasma-like organisms. *Phytopathology* 83:834-842.
- Lee, I.-M., M. Klopmeier, I.M. Bartoszyk, D.E. Gundersen-Rindal, T.-S. Chou, K.L. Thompson, and R. Eisenreich. 1997. Phytoplasma induced free-branching in commercial poinsettia cultivars. *Nature Biotechnol.* 15:178-182.
- Luby, J.J., J.F. Hancock, and J.R. Ballington, Jr. 1992. Collection of native strawberry germplasm in the Pacific Northwest and northern Rocky Mountains of the United States. *HortScience* 27:12-17.
- Maas, J.L. (ed.). 1984. *Compendium of strawberry diseases*. APS Press, St. Paul, Minn.
- Postman, J., R.R. Martin, and P. Catling. 1997. *Fragaria multicipita*—A pathogen-induced taxon. *Acta Hort.* 471: (In press.)
- Reed, C.F. 1966. Wild strawberry species of the world, p. 118. In: G.M. Darrow (ed.). *The strawberry, history, breeding, and physiology*. Holt, Rinehart and Winston, New York.
- Stuessy, T.F. 1990. *Plant taxonomy. The systematic evaluation of comparative data*. Columbia Univ. Press, New York.
- Tomlinson, P.B. 1984. Vegetative morphology—Some enigmas in relation to plant systematics, p. 49-66. In: V.H. Heywood and D.M. Moore (eds.). *Current concepts in plant taxonomy*. Systematics Assoc. Spec. Vol. No. 25. Academic, Orlando, Fla.
- Vibio, M., A. Bertaccini, I.-M. Lee, R.E. Davis, C. Minucci, and R.G. Milne. 1994. Etiology of lettuce yellows in Italy: Genetic characterization of associated mycoplasma-like organisms. *Phytopathol. Medit.* 33:179-186.