lations were greatly suppressed during the third month after the second application. Neither material produced adequate yield increases to justify use for nematode control.

The results of this work indicate that fern production can be improved significantly by programmed applications of nematicides such as Mocap, Furadan and Nemacur. Results also suggest that growers interested in establishing *P. penetrans*-free planting stock may consider programmed treatments of isolated stock beds with 30 lb/A Mocap, even though frond production may be suppressed temporarily. In this way nematode-free propagules could be produced as planting stock for new or refurbished ferneries.

Since pre-plant dipping of fern planting stock reduced but did not eliminate nematode infestations in the fern propagules, subsequent applications of residual, nonphytotoxic nematicides would be indicated even if pre-plant fern dips were used. Therefore, a maintainance program which includes incorporation of a residual nematicide into previously fumigated beds at time of planting and annually thereafter is suggested to prevent development of high nematode populations when plantings are established with nematode-infested stock.

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TEMPERATURE AND OTHER FACTORS AFFECTING THE FREQUENCY OF GALLING IN ARDISIA SEEDLINGS

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Abstract. **Factors such as temp, depth of planting, potting media, seed source, length of seed storage, seed preparation prior to planting, and time of year for seed harvest were studied in relation to their influence on frequency of galling in** Ardisia crenata.

A temp of 30 C (86 F) during seed germination was shown to significantly (P = 0.05) increase the frequency of galling over seed germinated at 25 C (77 F). Pregerminated seed treated at 35 C (95 F) for as little as 2 days increased galling significantly (P = 0.01) over non-germinated seed at the same temp. Potting media, seed source, time of year for seed picking, and depth of seed planting did not significantly (P = 0.05) influence gall formation.

The genus *Ardisia* of the family Myrsinaceae is comprised of several popular species grown as ornamental plants. *Ardisia crenata* Sims, the predominant species in Florida (K. R. Langdon, personal communication), is found outdoors as a woody shrub attractive for its shiny green foliage and clusters of red berries. Seedlings are grown commercially in greenhouses in Florida and other states for use mostly in terrariums and dish gardens.

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One of the most serious problems encountered in growing Ardisia seedlings in the nursery is stem galling which may affect up to 95% of the crop. A normal planting of Ardisia seedlings requires approximately 6 months or more of growing time prior to selling. Thus, valuable growing time and space can be lost when the gall problem is encountered. The galling in most cases appears first at the cotyledonary node, approximately 3 to 4 months after seed planting (Fig. 1). The axillary leaf buds at this node enlarge considerably from callus tissue accumulation and may reach a diameter of 0.5 cm or greater. A close examination of other nodes will also show enlargement of the axillary leaf buds. The galled plants develop dark green leaves (usually not more than 4) and remain stunted.

Repeated isolations and examinations of galled plants submitted to this laboratory over the past years have failed to reveal a pathogenic agent.

A review of the literature suggested that this problem may be similar to the gall formation induced in *Ardisia crenata* and *A. crispa* (Thunb.) A. DC. by the destruction of the foliar symbiotic relationship normally formed by the bacterium *Bacterium foliicola* (Meihe) DeJongh or *Chromobacterium lividum* (Eisenberg) Bergey et al. with the respective host plant (1,3,4,5,6,7). Either of these bacteria is apparently necessary to produce cytokinins for normal plant development (7). *B. foliicola* grows slowly at 32 C (5) and is killed after 1 to 2 days at 40 C (3,5,7). *C. lividum* is killed after 3 days at 40 C (7). Galling of *Ardisia* seedlings has been induced experimentally by exposing seed or seedlings at 40 C for 48 hours prior to planting (3,6). Symbiotic bacteria have not been detected in galled plants (3).

The purpose of this study was to determine what factors in Florida nurseries might contribute to the inducement of galling in *Ardisia crenata* placing special emphasis on temperature.

Materials and Methods

Ardisia seed were collected primarily from 2 sources: 1) Wilmot Gardens at the University of Florida, Gainesville with the cooperation of Dr. Willard T. Witte and 2) Ardisia Gardens, Saint Leo, Florida with the cooperation of Brother Fred Sherer. Voucher specimens were collected in October 1975 from these locations and were deposited by the senior author in the Florida Department of Agriculture and Consumer Services, Division of Plant Industry Herbarium at Gainesville.

The seed were planted generally within several days after collection, or air-dried for 1 week prior to storage at 10 C. At least 100 seed were plant-

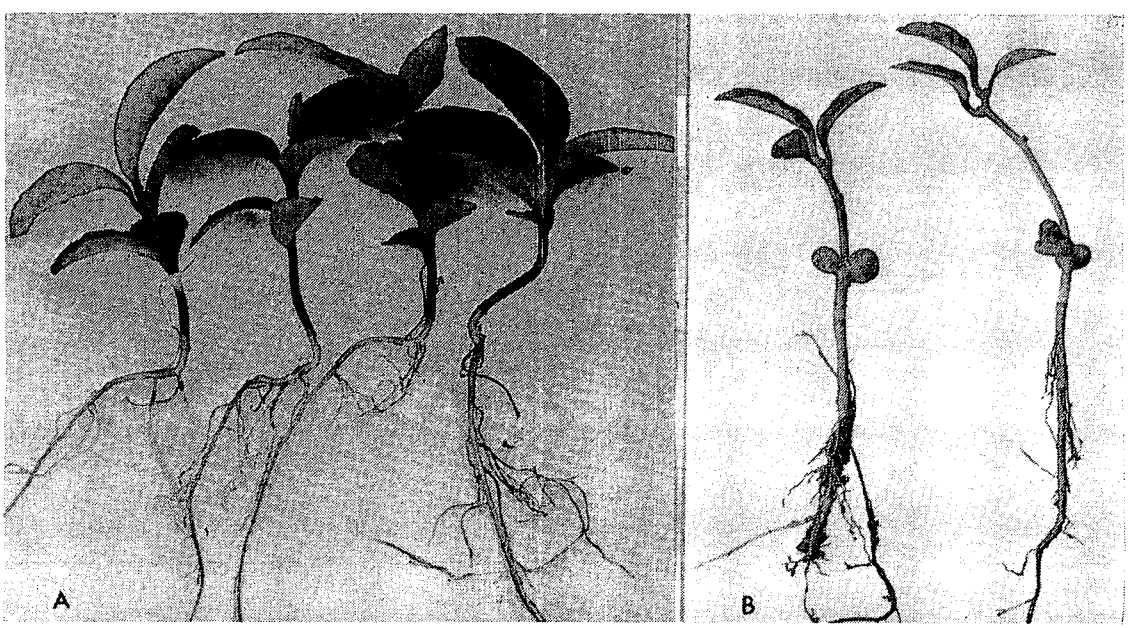

Fig. 1. Normal and galled 6-month-old seedlings of Ardisia. A) Normal, healthy seedlings. B) Galled plants showing large galls at cotyledonary node and small galls developing in leaf axils and at growing point.

ed for each treatment either with pulp intact, depulped, or pre-germinated from intact or depulped seed. Seed were pregerminated at 25 C and used for testing when the radicle was approximately 1.5-2.0 cm long. Seed germination was over 80% unless specified otherwise.

The sterilized potting media used for these tests consisted of either Canadian peat, vermiculite, or a combination of peat and builders sand (1:1 v/v). Sterilized 6-inch (15.2 cm) diameter clay pots were used for most tests.

Treatments consisted of planting seed at 25, 30, or 35 C in a growth chamber set at a high relative humidity (95% RH) prior to removal to an air-conditioned greenhouse (air temperature 20-30 C). Direct plantings of seed in an air-conditioned greenhouse or non air-conditioned greenhouse (air temperature 21-40 C) were also made. Soil temperature recordings in commercial nurseries were conducted with a maximum-minimum Taylor Brookwood outdoor thermometer (Taylor Instrument, Sybron Corporation, Arden, NC 28704).

Results

Seed Sources, Soil Temperature, Potting Media, and Depth of Planting

Duplicate plantings in air-conditioned and non air-conditioned greenhouses were made using intact seed collected March 1974 and April 1974 from the Wilmot and Saint Leo sources, respectively. One hundred seed were planted at 2 depths in sand-peat in 6-inch (15.2 cm) diameter pots. The shallow depth consisted of barely covering the seed whereas in the deeper planting the seed were placed at approximately 3/4 inch (1.9 cm). After 3 months in the non air-conditioned greenhouse, the plants were transferred to the air-conditioned greenhouse. Final data on galling were recorded 210 days after planting. No significant differences ($P = 0.05$) were noted between respective seed sources and depths of planting within each temperature regime (Table 1). However, a significantly ($P = 0.05$) greater amount of galling occurred with both seed sources and depths of planting at the higher temperature regime of 21-40 C (non air-conditioned greenhouse) compared to seed germinated at the lower temperature regime of 20-30 C (air-conditioned greenhouse).

At a commercial nursery in Apopka, 4-month-old *Ardisia* seedlings grown in a peat-perlite medium (1:2 v/v) at warm (27 C+) and cool (21 C) growing sites were examined for galls. After 4 months, 54% of 1173 seedlings showed galling from the warm site whereas only 8% of 739 seedlings had galls from the cool site. At 6 months the galled seedlings in the warm growing site had increased from 54 to 66%. No changes occurred in the percentages of galled seedlings from the cool site. In addition, a comparison of the percentage of galled seedlings occurring in 2 soil

Table 1. Effect of shallow and deep seed planting at 2 temperature regimes on gall formation by Ardisia seedlings after 210 days.

Temperature regime	Depth of planting[z]	Seed source[y]	Percent galling
Air-cond. ghse (20-30 C)	shallow	WG	13
		SL	2
	deep	WG	3
		SL	7
Non air-cond. ghse (21-40 C)	shallow	WG	85
		SL	68
	deep	WG	54
		SL	44
LSD(P = 0.05) = 40			
LSD(P = 0.01) = 59			

[z]Intact seed.

[y]WG = Wilmot Gardens, SL = Saint Leo.

media in the warm growing site after 4 months showed 86.2% of 1896 seedlings were galled in an Aphelandra-soil mix (1 part peat, 0.8 part vermiculite, 0.7 part sand, and 1.5 part cypress shavings) in contrast to 54.0% of 1173 seedlings in a peat-perlite medium (1:2 v/v).

To determine the extent of fluctuations in soil temperatures in nurseries growing *Ardisia*, recordings of maximum-minimum soil temperatures were made daily in 4 nursery planting sites during the planting period of March 28 to July 15, 1975. Soil temperature changes were recorded in raised benches at a shallow seed planting depth for all sites. Peace River peat, peat mixed with perlite, and Oxford peat covered with Canadian peat were the soil media involved. The maximum and minimum temperatures fluctuated from 30-36 C to 18-24 C.

Seed from 3 sources (Wilmot Gardens and Saint Leo collected September 1974 and Green Thumb Corporation, Apopka collected October 1974) were depulped and each planted in vermiculite, peat, and sand-peat in 6-inch (15.2 cm) diameter pots at 25 and 30 C in growth chambers. The seed from Wilmont Gardens and Saint Leo were removed to the air-conditioned greenhouse after 2 months while the seed from Green Thumb were removed after 1 month. Data recorded 6 months after planting showed significant increases ($P = 0.05$) in percent galling by all 3 seed sources exposed to 30 C (Table 2). No significant differences in galling were noted between respective seed sources within each temperature or among the potting media.

In a similar trial concerned with the influence of seed source on the frequency of galling, seed were collected January through March 1973 from 7 locations (commercial nurseries and directly from *Ardisia* plantings). One hundred seed from each source were planted in perlite in 6-inch (15.2 cm) diameter pots in a non air-conditioned greenhouse (air temperature 20-30 C). Four months after planting, no galling was noted. At 6 months, only 1 to 2% of the seedlings were galled.

Effect of Different Exposure Times on Seed at 35 C on Gall Production

Pregerminated and intact seed collected February and March 1974, respectively, from Wilmot Gardens were compared for their susceptibility to galling following different exposure times (days) at 35 C. Seeds were removed after treatment to an air-conditioned greenhouse and data recorded 8 months after planting. Significantly ($P = 0.01$) greater differences were noted for frequency of galling in pregerminated seed than for intact seed at 2 through 16 days at 35 C (Table 3). Significantly ($P = 0.05$) greater differences in frequency of galling between the check (non treated) and exposure treatments were noted as early as 2 days for the pregerminated seed and only after 16 days for the intact seed.

Effect of Exposure of Pregerminated Seed at 35 C on Incubation Time Prior to Gall Formation

Pregerminated seed collected February 1974 from Wilmot Gardens were exposed for varying lengths of time at 35 C prior to removal to an air-conditioned greenhouse. Galling was recorded at 60, 120, 180, and 240 days after planting. Gall

Table 2. Effect of temperature and seed sources on incidence of galling of Ardisia planted in 3 potting media after 180 days.

Seed source[z]	Temp (C)	Potting medium[y] and percent galling V	P	S-P
WG	25	2	0	1
SL		24	39	19
GT		7	11	5
WG	30	99	100	73
SL		90	100	96
GT		96	74	90

LSD(P = 0.05) = 30
LSD(P = 0.01) = 41

[z]WS = Wilmot Gardens, SL = Saint Leo, GT = Green Thumb.

[y]V = vermiculite, P = Canadian peat, S-P = sand-peat (1:1 v/v).

Table 3. Effect of different exposure times at 35 C on gall production by pregerminated and intact seed after 240 days.

Exposure (days) at 35 C	Percent galling[z] by intact seed	pregerm seed
0	2	4
1	9	21
2	0	45
4	12	56
8	7	80
16	41	96
36	79	100

LSD(P = 0.05) = 30
LSD(P = 0.01) = 42

[z]Based on average germination rate of 61%.

formation occurred earlier (120 days) and in greater frequency as the exposure time at 35 C was increased from 1 to 36 days (Table 4). Significant differences (P = 0.05) were noted between the percentages of galls recorded at 120 and 180 days for the 2, 4, and 8 day exposures. No differences were found in the frequency of galling recorded at 180 and 240 days.

Effect of Seed Storage at 10 C on Galling

To date, limited data on galling of seedlings germinated in the air-conditioned greenhouse from seed stored for 2 months and 1 year at 10 C indicate that very little galling occurs from seed stored 2 months while nearly 100% galling occurs from seed stored 12 months. Seed germination was very low (ca 5-10%) for seed stored 12 months. Additional tests are being conducted to determine the maximum safe length of storage.

Effect of Time of Year of Seed Harvest on Galling of Seedlings

To date, seed obtained and planted in March, September, and October 1974 from the Wilmot Gardens and Green Thumb seed sources have not indicated a corresponding increase in galling by the seedlings relative to harvest time. The percentage galling recorded 6 months after planting in the air-conditioned greenhouse varied from 2 to 8% in this test.

Discussion

The results of this study showed that temp is perhaps the single most important environmental factor in regulating the frequency of gall formation in *Ardisia* seedlings. Although high temperature (40 C) has been demonstrated to be associated with gall formation (3,6), this study has shown that a soil temperature as low as 30 C (86 F) significantly increased the frequency of galling in seedlings when seed were exposed for 30 to 60 days at this temperature. Additional tests concerned with the effect of temperatures between 25 and 30 C and tests involving fluctuating temperatures especially those found in the nurseries would be desirable.

The most susceptible developmental stage of *Ardisia* to temperature effects in this study occurred at seed germination as noted in Table 3.

Table 4. Gall formation by pregerminated seed exposed to 35 C for different time intervals.

Exposure (days) at 35 C	Days after planting and percent galling 60	120	180	240
0	0	0	4	4
1	0	0	21	21
2	0	12	45	45
4	0	15	56	56
8	0	56	80	80
16	0	88	96	96
36	0	90	100	100

LSD(P = 0.05) = 21
LSD(P = 0.01) = 29

Non-germinated seed appeared protected against high temperature (35 C) especially if the seed pulp was intact. The sudden increase in frequency of galling from intact seed exposed for 16 days at 35 C may be at least partially explained by the fact that seed germination can and probably does occur within this incubation time. The results of this test also helped to explain the relatively low frequency of galling found in intact seed harvested at different times of the year. Approximately 6 months from planting should be allowed to determine the extent of galling in a planting as shown from data in Table 4.

Although depth of planting did not significantly influence the frequency of galling, a trend toward less galling was noted in the deeper planting (Table 1). This trend might be explained by the heating effect of high ambient air temperature (30-40 C) at the soil surface especially in the presence of high humidity.

Although seed sources did not indicate significant differences in the frequency of galling, galling did vary according to the source (Table 2). Differences in frequency of galling (7-22%) by seed from 7 sources were reported by DeJongh (3), but no explanation for the variability was given.

Although the potting medium did not influence the frequency of galling under controlled air temperatures (Table 2), it did appear to be associated with differences in galling at a commercial nursery. Additional studies in regard to the influence of the potting medium may be desirable.

Based upon the results of this study, it appeared that the frequency of galling can be reduced significantly by maintaining soil temperatures at 25 C (77 F). Low soil temp can be obtained by (1) controlling the air temperature, (2) planting during a cool time of the year, or (3) maintaining good air circulation to maximize the cooling effects resulting from moisture evaporation from the soil surface. Maintaining adequate moisture levels in the soil medium as well as using a soil medium such as peat which has a high specific heat and resists a rapid temp change would be further advantageous to soil temperature control (2). Greenhouses having poor air circulation, high temperature, and high humidity would be undesirable for successful production of *Ardisia.*

Although the symbiotic bacteria necessary for normal development by certain *Ardisia* species were not investigated here, it appeared that the temperatures of 30 and 35 C (86 & 95 F) and length of exposures tested may well have interfered with the establishment of the bacterium or bacteria in the symbiotic relationship with *Ardisia crenata.*

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