Effects of Temperature, Moisture, and Stage of Inflorescence Development on Infection of Pineapple by *Penicillium funiculosum* and *Fusarium moniliforme* var. *subglutinans*

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ABSTRACT

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Data on interfruitlet corking (IFC), leathery pocket (LP), and fruitlet core rot (FCR) induced by *Penicillium funiculosum* were collected from 36 tests from 1972 through 1978 and on FCR induced by *Fusarium moniliforme* var. *subglutinans* from 26 tests. Correlation coefficients were calculated for the incidence and severity of infected fruit versus temperature, rainfall, and leaf wetness during the first 15 weeks following the chemical forcing (time postforce) of flower induction. With natural infection by *P. funiculosum*, significant positive correlations occurred between hours per week at 16–21 C and IFC, LP, and FCR during the first 5 weeks postforce. In contrast, with artificial inoculations, significant positive correlations occurred at 10–15 weeks postforce. With infections by *F. moniliforme* var. *subglutinans* in both natural and artificial inoculations, significant positive correlations occurred for hours per week at 21–27 C and FCR during flowering (10–15 weeks postforce). Rainfall was more important for natural infections than for artificial inoculations with *P. funiculosum*. Significant positive correlations occurred with natural infections during 1–5 weeks postforce and 10–15 weeks postforce. No rainfall correlation patterns were noted for infections by *F. moniliforme* var. *subglutinans*. Under a controlled environment, maximum infections by *P. funiculosum* (as indicated by unopened flowers) and IFC, LP, and FCR fruit symptoms occurred at 22 C day/14 C night. When inflorescences at different stages of development were subjected to 22 C day/14 C night, maximum infections occurred in the 1.25 cm open heart stage and in the late cone to early flower stage. No infection correlation patterns were noted in inoculations with *F. moniliforme* var. *subglutinans*.

Additional key words: *Ananas comosus*.

Interfruitlet corking (IFC), leathery pocket (LP), and fruitlet core rot (FCR) of pineapple fruit, *Ananas comosus* (L.) Merr., are caused by infection of the inflorescence by *Penicillium funiculosum* Thom. just prior to flowering or approximately 9–11 weeks following chemical forcing of flowering (12,14). Fruitlet core rot can also be induced by flower infections with *Fusarium moniliforme* Sheld. var. *subglutinans*, which previously was reported in Hawaii as *F. moniliforme* (8,13). Yeasts and bacteria also have been associated with FCR (13).

Certain environmental conditions have been associated with high incidences of IFC, LP, and FCR. For example, in Australia where *P. funiculosum* is associated with FCR (9), incidence is most severe in fruit from plants that flower in December (1). LP and FCR in South Africa occurs in fruit harvested in April–May from plants that flowered in October–December (6). In Brazil, where the rainy season occurs during the warm summer months, *F. moniliforme* var. *subglutinans* is associated with severe FCR (8). In Hawaii, high levels of disease have been associated with cool-wet conditions. Highest incidences of IFC, LP, and FCR due to infection by *P. funiculosum* occur in fruit differentiated in September–November and harvested in late spring to early summer (April–June) (5,12,13).

Inflorescence development progresses through several stages (Table 1) and in Hawaii requires about 106 days from initiation to completion of flowering (7). Inflorescence development of cultivar A is ~1 wk shorter than that of the commercial cultivar Smooth Cayenne and cultivar B. Depending on temperature, inflorescence emergence for cultivar A occurs from 5 to 7 weeks following chemical flower forcing (postforce).

These studies were undertaken to determine the optimum climatic conditions for infection of the pineapple inflorescence by *P. funiculosum* and *F. moniliforme* var. *subglutinans* in relation to inflorescence development stages. A preliminary report has been published (10).

MATERIALS AND METHODS

Pineapple culture and experimental design. Pineapple cultivar A, which is highly susceptible to *P. funiculosum*, and cultivar B, which is highly susceptible to *F. moniliforme* var. *subglutinans* (13), were grown according to standard cultural practices for pineapple in Hawaii (2). Fourteen field sites planted to cultivar A and nine sites planted to cultivar B were selected at various locations on the island of Oahu from 1972 through 1978. Groups of plants at each site were treated with ethephon (Union Carbide Agricultural Products Co., Inc., Ambler, PA 19002) to force flowering (3) on two to four occasions at 2- to 6-wk intervals ranging from 31 July to 15 December resulting in two to four separate tests at each location. Each test consisted of an inoculated (artificial infection) and uninoculated (natural infection) treatment and was replicated four times. Plot size varied from 6 to 14 fruits per replication. Inoculations were accomplished as previously described (13). A suspension of 10^7 spores of *P. funiculosum* per milliliter was sprayed into the differentiated growing point of each cultivar A plant, while 10^5 spores of *F. moniliforme* var. *subglutinans* per milliliter was sprayed on cultivar B plants prior to and during flowering. The number of inoculations in each test varied from one to three.

Climatic data. A 7-day recording thermograph, a 30-day recording rain gauge, and a 7-day Dewitt leaf wetness recorder were installed at each test site. Temperature data were summarized as the

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total number of hours per week in each of five temperature ranges (10–16, 16–21, 21–27, 27–32, and 32–38 C) and minimums and maximums. Rainfall was summarized as total centimeters per week and leaf wetness as hours per week.

Disease data. Fruit were harvested when 30–100% of the fruitlets were yellow. Harvest periods ranged from 7 February–7 March to 22 July–22 Aug. The incidence of IFC was recorded as the percentage of fruits per treatment with symptoms on the shell. Severity of IFC was recorded using the scale shown in Fig. 1. Incidence and severity of LP and FCR were recorded as for IFC after the removal of the shell.

Data analysis. All data were analyzed by using Statistical Analysis System (SAS) programs (SAS Institute Inc., Cary, NC). Temperature, rainfall, and leaf wetness data were coded to weeks of the year (weeks 1 to 52) for each year. Disease data were paired with climatic data so that weekly climatic data corresponded to that of the first 15 weeks postforce for each test. Correlation coefficients (Pearson) were computed for each climatic factor versus IFC, LP, and FCR by week following forcing. Inoculation frequency was examined together and independently with disease.

Controlled-environment studies. Crowns of cultivars A and B from Hawaii were grown at the Southeastern Plant Environment Laboratories at North Carolina State University, Raleigh, in an air-conditioned greenhouse at 26 C day/22 C night for 4 mo followed by type "B" growth chambers (4) at the same temperatures for 2 mo. Standard culture substrate, nutrient solutions, and watering schedules were used (4). Illumination, temperature control, relative humidity control, and CO_2 levels for B chambers were described by Downs and Bonamino (4), and included 12-hr, high-intensity light periods. Plants were forced to flower with ethephon following 6 mo of growth. Plant weights at forcing were ~1.12 kg for cultivar A and 0.93 kg for cultivar B.

Four different tests were conducted. In test I, 12 inoculated plants of cultivar A were placed in each of four "B chambers" (4) at day/night temperatures of 22/14, 22/18, 22/22, and 30/22 C, respectively. Subtreatments (six plants each) consisted of high humidity (wet cotton placed in the plant heart over the developing inflorescence and saturated twice daily when plants were watered) and ambient humidity (B chamber RH ranged from 40–70% day to 70–95% at night (4). Plants were held at the treatment temperatures for 6 wk then moved to the air-conditioned greenhouse at 30 C day/26 C night until fruit harvest.

In test II, inoculated plants at the inflorescence development stages of 1.25 cm open heart, 2.5 cm open heart, mid- to late cone, and late cone to early flower (Table 1) were exposed to 22 C day/14 C night for 5 days. Humidity subtreatments were as described above.

In test III, plants of cultivar B were treated the same as in test I except that inoculations were made by applying ~0.2 ml of a suspension containing 10^5 spores of *F. moniliforme* var. *subglutinans* per milliliter in the corolla of each new flower three times per week (flowers remain open only 1 day). Test IV, varied from test III only in that the inoculum used contained 0.01 and 1.0% Triton X-100 surfactant (Rohm & Haas Co., Philadelphia, PA 19105).

Data collection. In all growth chamber tests, data were collected weekly on the stage of inflorescence development. In tests I and II, the numbers of unopened, partly opened (tip of petals extending through the calyx), and opened flowers per inflorescence were recorded when flowering was completed. When fruit matured, data were collected on fruit weight, IFC incidence and severity, and the number of fruitlets showing LP and FCR. In tests III and IV, fruit weights and the number of fruitlets with FCR were recorded.

RESULTS

Field data correlations. Disease levels varied from 0–100% among forcing dates. Significant positive correlations occurred between numbers of hours per week at 16–21 C and IFC, LP, and FCR during the 4 weeks postforce in tests without inoculation with *P. funiculosum* (Fig. 2). Significant negative correlations of IFC and LP occurred several times throughout the 15-week postforce period with hours per week at temperatures from 27–38 C under uninoculated and inoculated conditions (Figs. 2 and 3). In contrast, significant positive correlations of IFC and LP occurred with hours per week at 16–21 C during the 10- to 15-week postforce period when they were inoculated (Fig. 3).

In tests with *F. moniliforme* var. *subglutinans* under both uninoculated and inoculated conditions, significant positive correlations occurred for 21–27 C with FCR during the 2- to 15-week postforce period. Significant negative correlations of FCR occurred with hours per week at 27–38 C during the later stages of inflorescence development (Figs. 2 and 3).

Without inoculation with *P. funiculosum*, significant

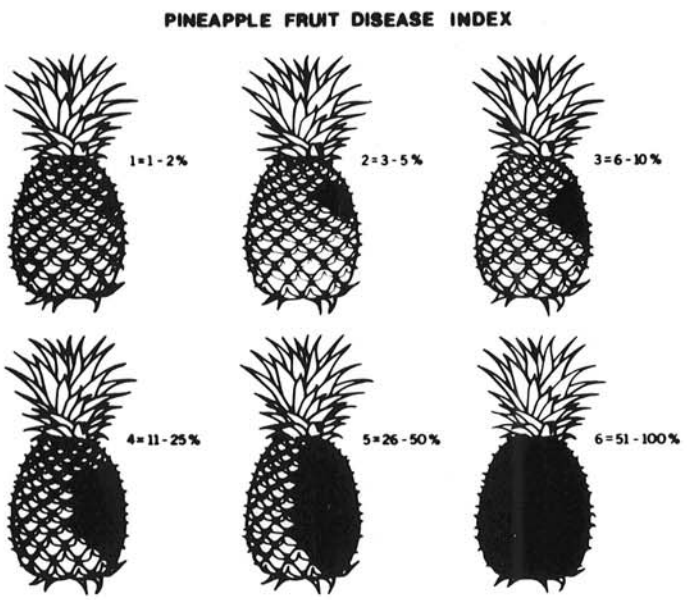

Fig. 1. Pineapple fruit disease scale for scoring severity of each symptom in which 0 = no fruitlets showing symptoms, 1 = 1–2% of the fruitlets with symptoms, 2 = 3–5%, 3 = 6–10%, 4 = 11–25%, 5 = 26–50%, and 6 = 51–100%.

TABLE 1. Stages of pineapple inflorescence development used by the pineapple industry to estimate forcing peaks and harvest dates

Code	Stage	Description
1	1.25 cm open heart	Growing point 1.25 cm open, exposing the emerging inflorescence
2	2.5 cm open heart	Growing point 2.5 cm open, exposing the emerging inflorescence
3	Early cone	One-third of flower buds visible in growing point
4	Mid cone	Two-thirds of flower buds visible in growing point
5	Late cone	All of flower buds visible in growing point
6	Early flower	Anthesis visible in lower one-third of inflorescence
7	Mid flower	Anthesis visible in middle third of inflorescence
8	Late flower	Anthesis visible in top third of inflorescence
9	Dry petal	Anthesis complete and petals dry

correlations occurred for rainfall at 3–4 and 12–14 weeks postforce for IFC, LP, and FCR. No meaningful pattern was noted for inoculation with *P. funiculosum* or with *F. moniliforme* var. *subglutinans* (Fig. 4).

No significant correlation patterns were noted for IFC, LP, or FCR with leaf wetness or minimum-maximum temperatures.

Controlled-environment tests. Cultivar A inflorescence development with day/night temperatures of 30/22, 22/22, 22/18, and 22/14 C reached stage 8–9 in about 12, 13, 14, and 15 wk, respectively; cultivar B reached stage 8–9 in about 12, 13, 13, and 14

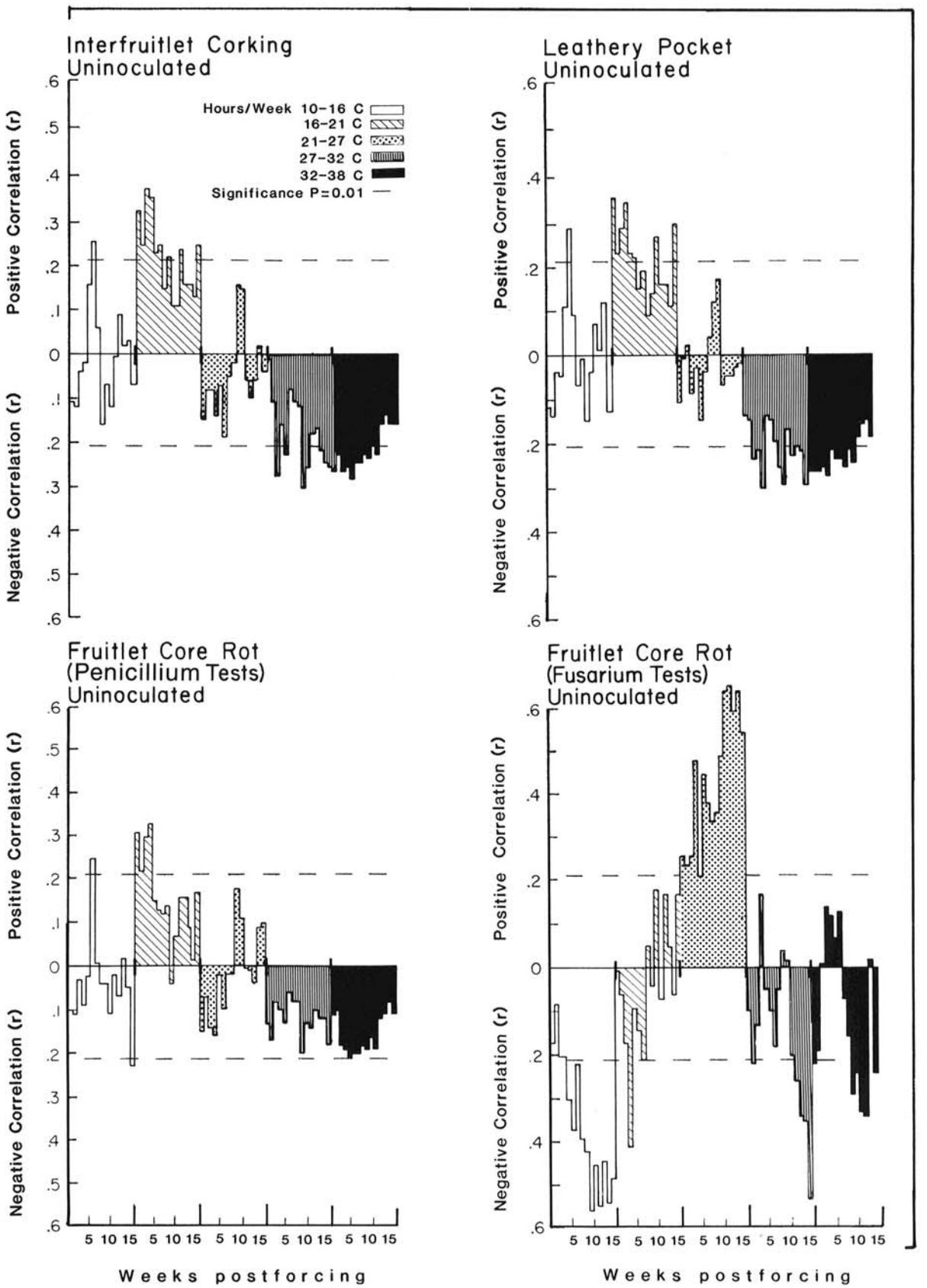

Fig. 2. Simple correlations by week postforce (1–15) of hours per week at 10–16, 16–21, 21–27, 27–32, and 32–38 C with interfruitlet corking, leathery pocket, *Penicillium funiculosum* fruitlet core rot and *Fusarium moniliforme* var. *subglutinans* fruitlet core rot without inoculation.

wk, respectively. Flower infections by *P. funiculosum* in cultivar A, as evidenced by unopened or partly opened flowers (5), and IFC symptoms at fruit maturity, were greatest at 22 C day/14 C night but were not statistically different among treatments (Table 2). Humidity level with varying temperature did not appear to affect disease development. Exposing inflorescences at different stages of development to 22 C day/14 C night for 5 days resulted in appreciable infections of inflorescences at the 1.25 cm open heart stage with low RH treatment and at the late-cone to early flower stage with high RH treatment (Table 3). Highest FCR in cultivar B

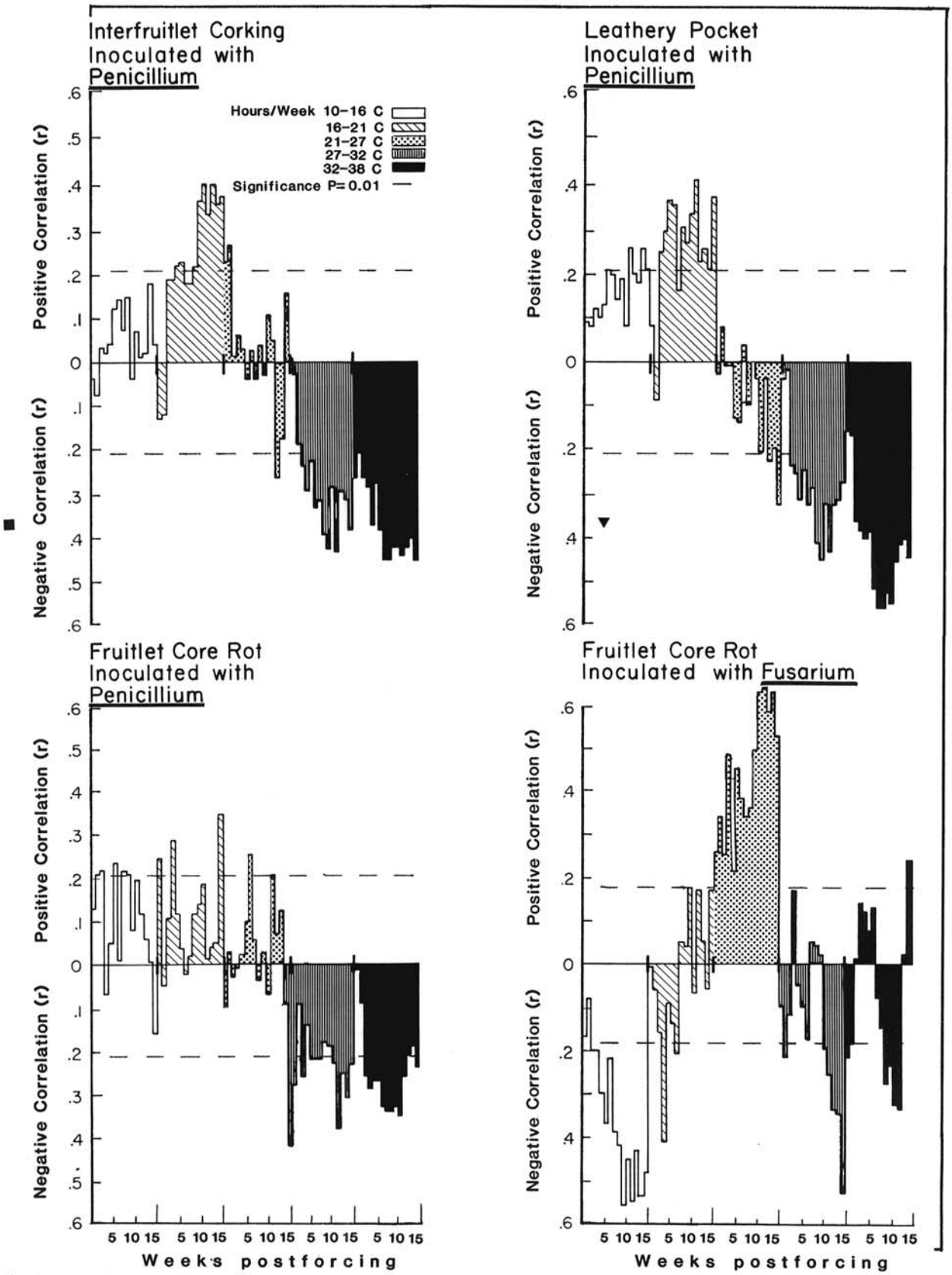

Fig. 3. Simple correlations by week postforce (1–15) of hours per week at 10–16, 16–21, 21–27, 27–32, and 32–38 C with interfruitlet corking, leathery pocket, *Penicillium funiculosum* fruitlet core rot and *Fusarium moniliforme* var. *subglutinans* fruitlet core rot with inoculation.

inoculated with *F. moniliforme* var. *subglutinans* occurred at 30 C day/22 C night in test III (Table 4) and 22 C day/18 C night in test IV (Table 5). No significant differences in FCR occurred between moisture level or surfactant level.

Fruit weights of cultivar A were significantly reduced with the high levels of disease at the long low-temperature exposure but not with the lowest disease levels at the shorter (5-day) low-temperature exposure (Table 2 versus 3). In contrast, cultivar B fruit weights were not significantly affected by the lower temperature exposures (Tables 4 and 5).

Coefficients of variation (CV) for IFC, FCR, and closed flowers ranged from 86 to 309% while CVs for total flowers per inflorescence and fruit weights were 10–22%. In contrast, CVs for IFC and FCR in the field correlation studies were generally <25% and frequently <10%.

DISCUSSION

In general, tests of *P. funiculosum* with August forcings had low levels of disease while tests of *F. moniliforme* var. *subglutinans* had high levels. The positive correlations of IFC, LP, and FCR caused by *P. funiculosum* at the maximum numbers of hours per week at 16–21 C coincides with the observations of Hepton and Anderson (5) that cool conditions during inflorescence development are associated with high levels of IFC. Without inoculation with *P. funiculosum*, the critical environmental period is during the 5 weeks postforce when inoculum level must build up for infection of the developing flower. When high levels of inoculum of *P. funiculosum* are placed in the plant heart during the 5-week postforce period, the importance of environment is lessened. Positive correlations of IFC and LP with hours per week at 16–21 C occurred at 10–15 weeks postforce, at the late cone to early flower stages. It is at these stages when infection of the internal flower parts (anthers, style, placental, and nectary tissues) actually occurs (14).

Positive rainfall correlations with IFC, LP, and FCR at 3–4 and 12–14 weeks postforce without inoculations with *P. funiculosum* indicate the importance of moisture in the pineapple plant heart for inoculum buildup and infection. Thus inoculum of *P. funiculosum* or inoculum potential must build up in the plant heart prior to actual infection of the inflorescence which is initiated at 5–7 weeks postforce. High artificial inoculum levels at this time negate the low

TABLE 2. Effects of four temperature regimes during pineapple inflorescence development on flower opening, interfruitlet corking (IFC) and its severity (S), and fruitlet core rot (FCR) of cultivar A fruit inoculated with *Penicillium funiculosum*

Day/night temp (C)	Flowers infected per inflorescence (mean no.) Unopened	Partly opened	IFC %	IFC S	FCR (mean no./ fruit)	Fruit weight (mean g)
22/14	28	11 a[z]	100	2.3	0.0	1,172 b
22/18	19	7 ab	33	1.0	1.0	1,263 b
22/22	1	2 b	33	1.5	0.5	1,678 a
30/22	0	2 b	17	1.0	0.2	1,540 a

[z]Within columns, means not followed by letters are not significantly different, and means followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

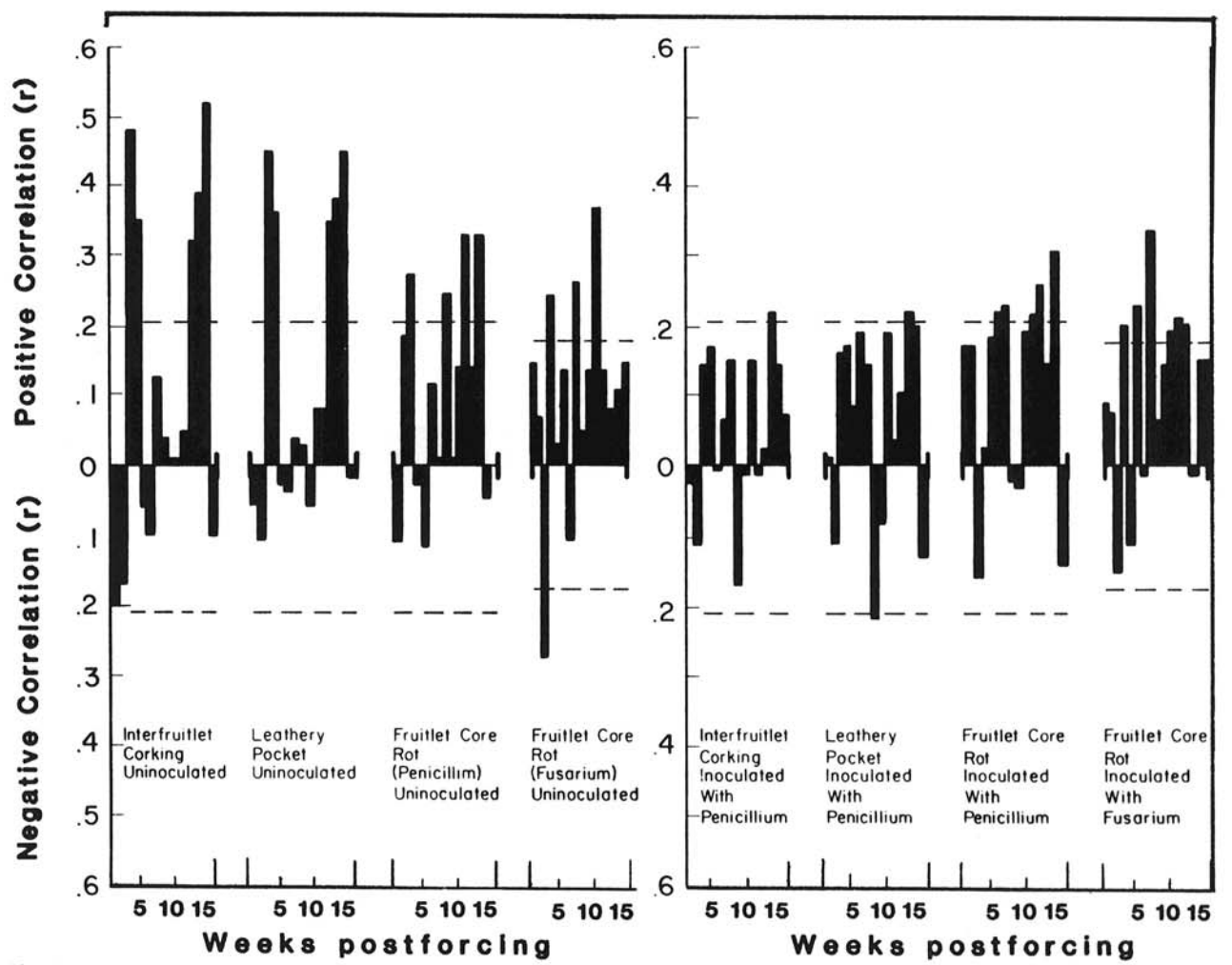

Fig. 4. Simple correlations by week postforce (1–15) of cm rainfall per week with interfruitlet corking, leathery pocket, and fruitlet core rot induced by *Penicillium funiculosum* or *Fusarium moniliforme* var. *subglutinans* under uninoculated and inoculated conditions.

TABLE 3. Effects of a 5-day, 22 C day/14 C night temperature exposure and two humidity levels at different stages of pineapple inflorescence development on flower opening, interfruitlet corking (IFC) percentage and severity (S), leathery pocket (LP), and fruitlet core rot (FCR) of cultivar A fruit inoculated with *Penicillium funiculosum*

Inflorescence stage[y] (code no.)	RH	Flowers infected per inflorescence (mean no.)		IFC		FCR (mean no./ fruit)	Fruit weight (mean g)
		Unopened	Partly opened	%	S		
Code 1	Ambient	12.0 ab[z]	8.8	16	2.0	3.3	1,237
	High	1.5 bc	5.7	16	1.0	3.0	1,405
Code 2	Ambient	0.3 c	1.0	0	0.0	0.8	1,187
	High	0.3 c	2.7	0	0.0	2.0	1,165
Code 4 to 5	Ambient	0.2 c	2.3	16	2.0	2.3	1,230
	High	4.3 abc	3.0	16	2.0	1.8	1,410
Code 5 to 6	Ambient	0.2 c	4.7	16	1.0	0.0	1,087
	High	13.0 a	8.2	0	0.0	2.3	1,170

[y] For coded names of inflorescence development stages, see Table 1.
[z] Within columns, means not followed by letters are not significantly different, and means followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 4. Effects of four temperature regimes at ambient RH on fruitlet core rot (FCR) of pineapple cultivar B fruit inoculated with *Fusarium moniliforme* var. *subglutinans*

Day/night temp (C)	FCR (mean no./ fruit)	Fruit weight (mean g)
22/14	2.0 ab[z]	1,087
22/18	2.5 ab	890
22/22	1.5 b	1,064
30/22	5.5 a	943

[z] Within columns, means not followed by letters are not significantly different, and means followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 5. Effects of four temperature regimes on fruitlet core rot (FCR) of cultivar B pineapple fruit inoculated with *Fusarium moniliforme* var. *subglutinans*[y]

Day/night temp (C)	FCR (mean no./ fruit)	Fruit weight (mean g)
22/14	4.3 ab[z]	996
22/18	8.2 a	907
22/22	2.3 ab	639
30/22	>1.0 b	629

[y] Inoculum suspension 0.2 ml containing 10^5 spores per milliliter and 1.0% Triton X-100 surfactant.
[z] Within columns, means not followed by letters are not significantly different, and means followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

temperature and high moisture requirement. Recent incomplete studies (11) have implicated the pineapple fruit mite *Steneotarsonemus ananas* in the disease development caused by *P. funiculosum*. Observations indicate that mite populations must be high just prior to forcing and 4–5 weeks postforce for disease development. The observed preference for cool-wet conditions by this mite would involve it in the positive correlations from forcing to 5 weeks postforce.

Correlation patterns of temperature with FCR induced by *F. moniliforme* var. *subglutinans* clearly show that the number of hours in the 21–27 C temperature regime is critical during flowering. This would explain the general prevalence of this disease in the warmer-wetter areas of pineapple production such as Brazil and Honduras (8, and *unpublished*) or seasonal differences where flowering occurs under these conditions. Rainfall correlation patterns were not clearly defined. In Hawaii, the relative importance of *F. moniliforme* var. *subglutinans* as an FCR pathogen varies with the season (13). The predominance of *P. funiculosum* as the FCR pathogen in the fall-winter forcings of the tests of *F. moniliforme* var. *subglutinans* may in part explain the lack of clear correlation patterns.

Controlled-environment studies generally confirmed field observations. However, statistical differences usually were not significant. CV for disease parameters were very high in contrast to plant characteristics such as fruit weights and total flower numbers. Field disease CVs are low since multiple plants are used in each replication. Therefore, disease variability between plants limits the use of controlled-environment facilities where plant numbers must be limited. The high plant to plant variation of mite populations recently observed may account for the high disease variation.

LITERATURE CITED

1. Anonymous. 1975. The Pineapple. Hortic. Branch, Dep. Primary Industry, Queensland, Australia. 32 pp.
2. Collins, J. L. 1960. The Pineapple. Interscience, New York. 294 pp.
3. Cooke, A. R., and Randall, K. I. 1968. 2-Haloethanephosphonic acid as ethylene releasing agents for the induction of flowering in pineapple. Nature 281:974.
4. Downs, R. J., and Bonamino, V. P. 1976. Phytotron procedural manual for controlled environment research at the Southeastern Plant Environment Laboratories. N. C. Agric. Exp. Stn. Tech. Bull. 244. 37 pp.
5. Hepton, A., and Anderson, E. J. 1968. Interfruitlet corking of pineapple fruit, a new disease in Hawaii. Phytopathology 58:74-78.
6. Keetch, D. P. 1977. H.1 Black spot (fruitlet core rot) in pineapples. Pineapple Series H. Diseases and Pests. Republic of South Africa, Govt. Printer, Pretoria. 3 pp.
7. Kerns, K. R., Collins, J. L., and Kim, H. 1936. Developmental studies of the pineapple: *Ananas comosus*: (L.) Merr. I. Origin and growth of leaves and inflorescence. New Phytol. 35:305-317.
8. Laville, E. 1980. La Fusariose de l'ananas au Brésil. I-Synthése des connaissances actuelles. Fruits 35:101-113.
9. Oxenham, B. L. 1962. Etiology of fruitlet core rot of pineapple in Queensland. Queensl. J. Agric. Sci. 19:27-31.
10. Rohrbach, K. G. 1980. Climate and fungal pineapple fruit diseases. (Abstr.) Page 60 in: Proc. Second Southeast Asian Symposium on Plant Diseases in the Tropics, 20–26 October 1980, Bangkok, Thailand.
11. Rohrbach, K. G., Namba, R., and Taniguchi, G. 1981. Endosulfan for control of pineapple interfruitlet corking, leathery pocket, and fruitlet core rot. (Abstr.) Phytopathology 71:1006.
12. Rohrbach, K. G., and Pfeiffer, J. B. 1976. Field induction of pineapple interfruitlet corking, leathery pocket, and fruitlet core rot with *Penicillium funiculosum*. Phytopathology 66:392-395.
13. Rohrbach, K. G., and Pfeiffer, J. B. 1976. Susceptibility of pineapple cultivars to fruit diseases incited by *Penicillium funiculosum* and *Fusarium moniliforme*. Phytopathology 66:1386-1390.
14. Rohrbach, K. G., and Pfeiffer, J. B. 1976. Infection of pineapple flowers by *Penicillium funiculosum* in relation to inflorescence development. (Abstr.) Proc. Am. Phytopathol. Soc. 3:231.