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BEAN PLANT PROTECTION

WORKSHOP



PROGRAM [ray + sent]

December 1 - 3, 1975



BIBLIOTECA

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CENTRO INTERNACIONAL DE AGRICULTURA TROPICAL (CIAT)

P R O G R A M

Monday, December 1 - Joint Session - Room "A"

08 00 - 08 30	Registration	
08 30 - 08 45	Words of welcome	<u>Fernando Fernández</u>
08 45 - 09 15	CIAT's Bean Program	<u>Peter H. Graham</u>
09 15 - 09 30	Coffee break	
09 30 - 12 30	Visit to CIAT's field experiment plots	
12 30 - 13 30	Lunch	
13 30 - 14 00	Virus diseases of beans in Latin America, as limiting factors in bean production	<u>Rodrigo Gámez</u>
14 00 - 14 30	Identification and biology of whiteflies as vectors of bean virus diseases.	<u>Louise Russell</u>
14 30 - 15 00	Discussion	
15 00 - 15 15	Coffee break	
15 15 - 15 45	Bean Golden Mosaic and Bean Chlorotic mottle viruses - their transmission and control	G. Gálvez (slides) J. Bird <u>Alvaro Santos Costa</u>
15 45 - 16 15	Discussion	
16 15 - 16 45	Control of whiteflies, vectors of BCMV and BCMV	<u>Freddy Alonso</u>
16 45 - 17 15	Discussion	
18 00 - 19 00	Informal cocktail party	

Tuesday, December 2 - Joint Session - Room "A"

	Chairman <u>Rodrigo Gámez</u>	
08 15 - 08 45	Bean Rust Mosaic and related viruses and their transmission by beetles	<u>Howard Scott</u>
08 45 - 09 15	Discussion	

09 15 - 09 30	C o f f e e b r e a k	
09 30 - 10 00	Breeding for resistance to the various strains of Common Bean Mosaic Virus.	<u>Felco Drijfhout</u>
10 00 - 10 30	Discussion	
10 30 - 11 00	Use of serology for the identification of bean viruses in the field.	<u>Sue Tolin</u>
11 00 - 11 30	Discussion	
11 30 - 12 30	General discussion	
12 30 - 14 00	L u n c h	

Plant Pathology Session - Room "A"

	Chairman <u>Kazuhiro Yoshii</u>	
14 00 - 14 30	Survival of phytopathogenic bacteria in the tropics.	<u>Max Schuster</u>
14 30 - 15 00	Discussion	
15 00 - 15 15	C o f f e e b r e a k	
15 15 - 15 45	Screening for resistance to Bacterial Blights.	<u>Alfred Saettler</u>
15 45 - 16 15	Discussion	
16 15 - 16 45	Seed-borne diseases of soybeans and beans, and their control	<u>James Sinclair</u>
16 45 - 17 15	Discussion	

Entomology Session - Room "F"

	Chairman <u>Alfredo Saldarriga</u>	
14 00 - 14 45	Pests of beans in Latin America, with emphasis on cultural and biological control	<u>Leonce Bonnefil</u>
14 45 - 15 15	Discussion	
15 15 - 15 30	C o f f e e b r e a k	
15 30 - 16 15	The importance of <u>Empoasca</u> species as bean pests, their biology and control.	<u>Floyd Smith</u>
16 15 - 17 00	Discussion	

Wednesday, December 3

Plant Pathology Session - Room "A"

Chairman Eddie Echandi

08 15 - 09 00	Fungal diseases of beans, at different ecologies, in the tropics	<u>Eddie Echandi</u>
09 00 - 09 30	Discussion	
09 30 - 09 45	C o f f e e b r e a k	
09 45 - 10 15	Discussion on Rust.	
10 15 - 10 45	Discussion on Anthracnose	
10 45 - 11 15	Discussion on web-blight	
11 15 - 11 45	Discussion	
11 45 - 12 30	Discussion on other fungal diseases.	
12 30 - 14 00	L u n c h	

Entomology Session - Room "B"

Chairman Diego Nivas

08 15 - 08 45	Bean pod weevil and its control by resistant varieties	<u>José Mancía</u>
08 45 - 09 15	Discussion	
09 15 - 09 30	C o f f e e b r e a k	
09 30 - 10 00	The Mexican bean beetle and its control by resistant varieties	<u>P. S. Benepal</u>
10 00 - 10 30	Discussion	
10 30 - 11 00	The bean leafhopper and its control by resistant varieties	<u>Aart van Schoonhoven</u>
11 00 - 12 00	Discussion	
12 30 - 14 00	L u n c h	

Joint Session - Room "A"

Chairman Peter Cribb

14 00 - 15 00	Future cooperative research projects in entomology	
15 00 - 15 15	C o f f e e b r e a k	
15 15 - 16 15	Future cooperative research projects in plant pathology	
16 15 - 17 15	General discussion	
17 15 - 18 15	Closing words	<u>Eduardo Alvarez-Luna</u>

SEMINARIO SOBRE PROTECCION VEGETAL DEL FRIJOL (Phaseolus vulgaris)

BEAN (Phaseolus vulgaris) PROTECTION WORKSHOP

C.I.A.T

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ESTUDIOS EN Phaseolus vulgaris L. SOBRE EL CONTROL DE LA MOSCA BLANCA Bemisia tabaci (Genn) EN LA ZONA SUR-ORIENTE DE GUATEMALA.

Por: Freddy Alonzo, Ing. Agr. Fit.; M.Sc.
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Introducción

Los principales problemas entomológicos del frijol Phaseolus vulgaris L. en la zona sur-oriente del país lo constituyen -- en orden de importancia la chicharrita verde Empoasca sp, el picudo de la vaina Anthonomus grandis (Wagn) y la mosca blanca -- Bemisia tabaci (Genn), por estar presentes como plagas en casi todas las áreas en donde se cultiva esta leguminosa.

Considerando la importancia de la mosca blanca por su distribución y daño económico causando al frijol en ésta zona, el proyecto de entomología ha pretendido encontrar, a corto plazo, un control químico adecuado y económico. En la búsqueda de un control integrado, ha tratado de conocer la fluctuación de las poblaciones de éste insecto en el transcurso del año de acuerdo con las diferentes condiciones climáticas prevalentes y con la presencia de hospederos. Como una promesa para reducir el uso de pesticidas, también se ha propuesto identificar fuentes de resistencia en germoplasma nacional y proveniente de otros centros de investigación.

Control Químico de B. tabaci

Mediante la evaluación de insecticidas que hasta ahora se reportan en algunos países como los más efectivos en el control de la mosca blanca y la chicharrita verde (Bonnetil L. 1965; Cernell y Soto, 1970; Ambroz J, 1971; Díaz G., 1971a, Díaz, 1971b, Ramirez J., 1971; Lancía, Díaz y Molina, 1973; Schoonhoven A, 1975), se realizaron en el año 1974 ensayos replicados en estaciones experimentales de I.C.T.A. localizadas en Monjas e Ipala, Guatemala (En este artículo se presentan solamente los resultados obtenidos en Monjas).

Los estudios consistieron en la evaluación de nueve insecticidas espolvoreados a los 15 y 30 días después de la siembra y también en la evaluación de tres insecticidas granulados aplicados

cados en cinco épocas diferentes (al momento de la siembra, 7, 14, 21 y 28 días después de la misma). Los diseños experimentales usados fueron respectivamente el de bloques al azar simple y el de un arreglo combinatorio distribuido también en bloques completos al azar.

La siembra se hizo con la variedad Negro Jalpataqua a 0.4 m. entre surcos y a 0.2 m. entre plantas (2 granos por mata), previamente fertilizado con 195 Kg por Ha de 16-20-0.

En ambos experimentos se tomó como índice de eficiencia de control, la frecuencia de plantas con mosaico dorado. Las dosis usadas por hectarea, de los insecticidas evaluados en ambos ensayos se presentan en la Tabla 4.

Resultados y Discusión:

En base a la menor frecuencia de plantas (Tabla 1), con mosaico dorado se encontró en la evaluación de insecticidas asperjados, que Metasistox, seuido de Iuvacron y Folimat dieron la mejor protección contra mosca blanca con 3, 8 y 12 plantas enfermas respectivamente por parcela de 12.8 m².

Debe notarse que el mejor rendimiento (Tabla 1) se registró con Letasistox (854 Kg/Ha), que fué el tratamiento que tuvo la frecuencia menor de plantas con mosaico dorado.

Respecto a la evaluación de tres insecticidas granulados y de cinco épocas diferentes de aplicación para el control de las principales plagas del frijol, se encontró (Fig.1 y Tabla 2), que Furadan y Thimet dieron la mejor protección contra mosca blanca, siendo esta superior cuando la aplicación se hizo al fondo del surco al hacer la siembra (11 y 14 plantas con mosaico dorado por parcela de 16 m² respectivamente). Tiodan (al igual que el testigo), tuvo mucho mayor frecuencia de plantas con mosaico dorado y una tendencia no definida por épocas de aplicación.

En relación a la interacción insecticidas-épocas de aplicación para rendimiento (Fig.2 y Tabla 3), se encontró que Furadan y Thimet aplicados al momento de la siembra, fueron los mejores tratamientos con rendimientos de 518 y 429 kilogramos por hectarea, respectivamente.

Los rendimientos reportados en ambos ensayos (asperjados y granulados), son bajos debido a que el picudo de la vaina - A. godmani - merió los rendimientos en un 21 y en un 70 % respectivamente (Tablas 1 y 3). Este insecto no se controló,

TABLA 1.- RENDIMIENTO POR UNIDAD Y RECOLECCIÓN DE GRANO
CON O DORADO REGISTRADO CC. LOS INSPECTORES
DAS. S, CAL. (JUN.-AG.) 1970.

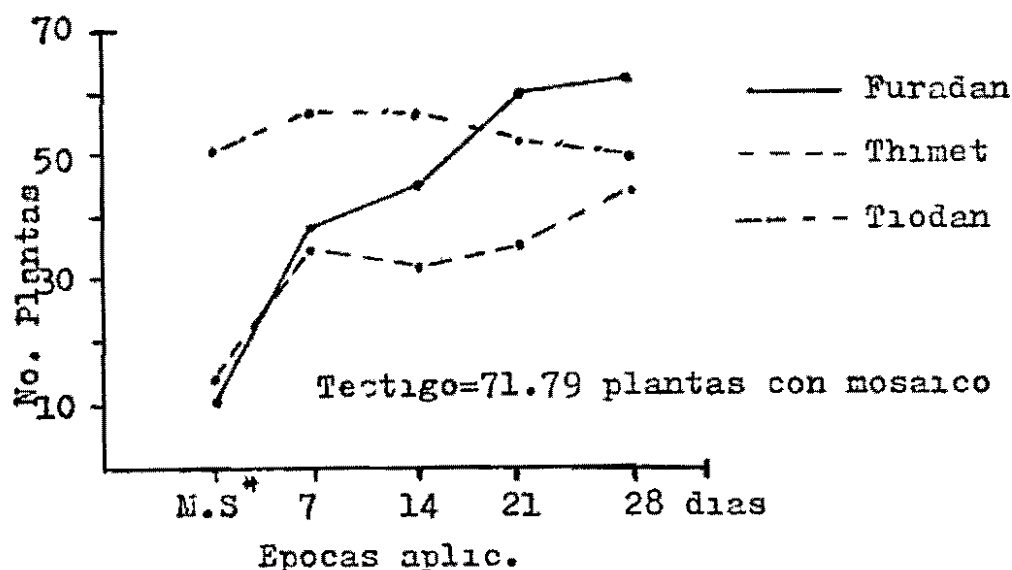
ER. TAMAÑO	Kg/Ha	No. PLANTAS POR TONELADA
MTASISION R-25	854	3
NUVACION - 50	620	2
FOLINAT - 80	581	12
SIVIT P.H.- 75	528	30
CARBITION - 100	479	11
DIPTEREX S.P. - 95	452	11
TIODIA 35 E.C.	425	11
TAMERON - 60	381	26
DELCORON - 100	346	30
TESTIGO (AGUA)	344	40
TESTIGO (NADA)	246	44

$\bar{X}$ PROMEDIO POR UNIDAD DE AREA POR HA DE = 12.8 m^2

$\chi^2 = 101.82$ $\chi^2_{0.05} = 23.21$ - -

PROMEDIO DE GRANO DAÑADO POR la recolección = 21,6

Figura 1.- Tendencia de la frecuencia promedio de plantas con mosaico dorado (en 16 m²) en cinco épocas - de aplicación de tres insecticidas granulados. Monjas, Jal. (Sep.-Dic.) 1974.



* Aplicacion al momento de la siembra

Tabla 2.- Discriminación de promedios de plantas con mosaico dorado (en 16 m²) en cinco épocas de aplicación de tres insecticidas granulados. Monjas, Jal. (Sep-Dic) 1974.

Epocas	Insecticida			Prom/ época
	Thimet	Furadan	Tiodan	
Al momento de la siembra	14	11	50	25
7 días después	34	36	57	43
14 días después	33	44	57	44
21 días después	36	59	52	49
28 días después	45	61	50	52
Promedio por Insecticida	32	42	53	
Promedio del Testigo	69.78			

Diferencias para insecticidas, para épocas y para la interacción **

Nota: Datos redondeados.

Figura 2.- Tendencia de rendimiento promedio de tres insecticidas granulados aplicados al suelo en cinco épocas de aplicación contra B.tabaci y Empoasca sp. Nonjas, Jal. (Sep.-Dic.) 1974.

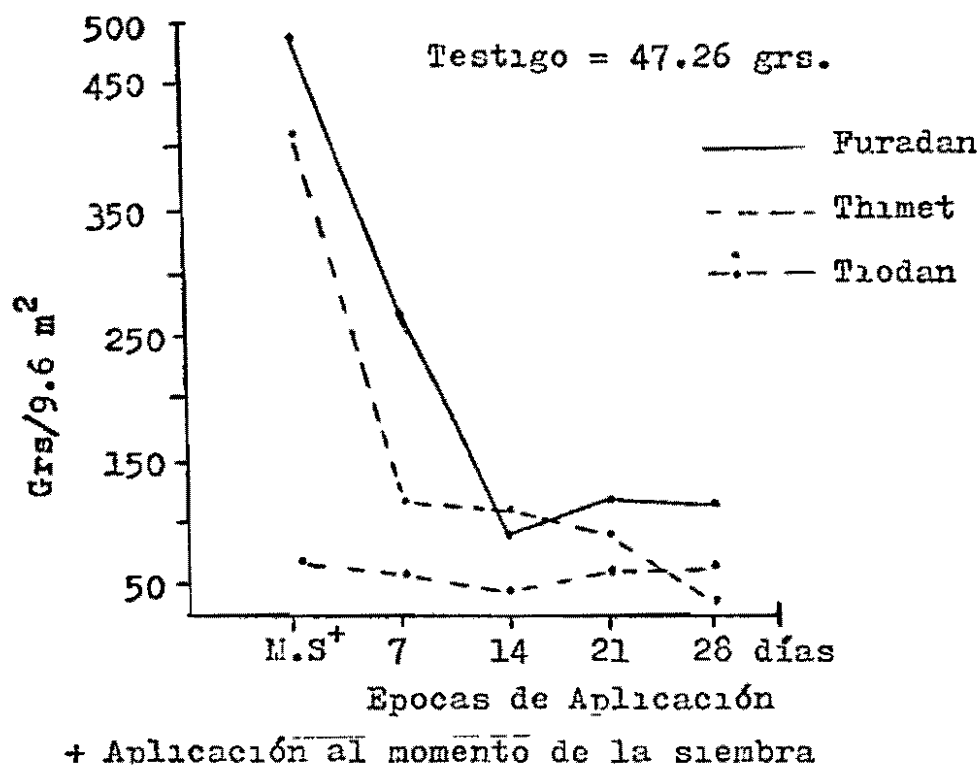


Tabla 3.- Discriminación de promedios de rendimiento (Kg/Ha) dados por los insecticidas granulados evaluados contra Bemisia y Empoasca en cinco épocas de aplicación. Nonjas, Jal. (Sep.-Dic.) 1974.

Epocas	Kg/Ha			Prom/Epoca
	Thimet	Furadan	tiodan	
Al momento de la siembra	429	518	81	411
7 días despues	174	214	54	176
14 días despues	107	122	42	107
21 días despues	74	124	72	97
28 días despues	33	93	60	84
Promedio por Insecticida	163	213	62	
Promedio del Testigo	49			

Diferencias para insecticidas, para épocas y para la interacción + +

Grano dañado por A. godmani = 69.6%

Nota: Datos redondeados

por convenir mas hacer análisis de residualidad en el grano, de los insecticidas aplicados, pudiendo la asperción de otro, alterar la residualidad original. En relación a ésto; se encontró (Tabla 4), que 30 días despues de la cosecha, tanto los insecticidas asperjados como los granulados aplicados al momento de la siembra, su residualidad fué minima (con excepción de Sevin que tuvo 0.132 ppm y de Folimat que tuvo 0.1 ppm); tal es el caso de Metasistox, de Furadan y Thimet que fueron los insecticidas que dieron mejor protección contra mosca blanca.

Debido a que el pequeño agricultor de esta zona labora generalmente en terrenos con pendientes mayor de 8 %, el autor considera que las razones por las que los insecticidas granulados le son, más promisorios que los asperjados son las siguientes: 1.- Alteran menos el equilibrio ecológico, permitiendo así que las poblaciones se mantengan a un nivel más bajo; 2.- El trabajo de aspersión en estos terrenos es muy difícil y muchas veces imposible; 3.- Se dificulta la obtención y transporte de agua para asperjar y 4.- Bajo la condición de lograrse una mezcla homogénea, pueden aplicarse juntamente con el fertilizante, reduciéndose así, el costo de aplicación.

Fluctuación de Poblaciones de *B. tabaci*

Estudios realizados con otros insectos por varios investigadores (Williams 1940; Kerkl y Perimer 1955, Hardino 1961; Wilde 1962, Kieckhefer et al 1964, Ship y Earhart 1967; Snow et al 1968, Johnson 1969, Leigh et al 1970, Sparks A. 1972; y Alonso 1973 a,b), señalan la importancia que tienen las condiciones climáticas prevalentes, así como el tipo de trampa, la altura y orientación de la misma, para incrementar la captura.

Kound (1962), en un estudio sobre la sensibilidad olfatoria y a colores de la mosca blanca, encontró que *B. tabaci* es atraída por dos grupos de luces, el azul ultravioleta y por el espectro de cartones amarillos. Amaya (1973), estudiando la atracción de la mosca blanca a diferentes colores usando como adherentes capa de vaselina y aceite lubricante l.o. 30, reporta que el amarillo 2601, es el color que más las atrae. También indica que las tarjetas de cartulina colocadas sobre o al nivel del follaje del frijol, tuvieron mayor captura.

TABLA 4.- RESIDUO LÍQUIDO (ppm) DETECTADO EN LA SEMILLA DE FRIJOL ALICHO 30 DÍAS DESPUÉS DE LA COSECHA.

INSECTICIDA	DOSES/ha	ppm
METASISLON R-25	1.00 lt	LD
NUVACRON - 50	1.50 lt	0.056
FOLIMAT - 80	0.33 lt	0.100
SEVIN S.P.-75	1.35 lt	0.132
CARBICRON -100	1.00 lt	ND
DIPTEREX S.P.-95	0.75 kg	ND
TIODAK 35-E.C.	1.50 lt	LD
TALLERON - 600	1.00 lt	0.051
DILICRON - 100	0.70 lt	ND
FURADAN - 10G	20.00 kg	ND
THIMET - 10G	20.00 kg	ND

ND = NO DETECTABLE

1/ ANALISIS REALIZADOS POR EL LABORATORIO UNIFICADO DE CONTROL DE FERTILIZANTES, MINISTERIO DE SALUD PÚBLICA, - DIRECCIÓN GENERAL DE SERVICIOS DE SALUD. CUAT.

En un intento por perfeccionar el método para estudiar fluctuación de poblaciones de mosca blanca, se estudiaron bajo condiciones de campo (0.5 Ha sembrado con la variedad Jama-pa), en Iongas, Jalapa las diferencias entre el empleo de tres formas de trampas (rectangulares, cúbicas y cilíndricas), a 15 alturas distintas (de 0.1 hasta 4 m), previamente pintadas con amarillo 2501. También se trató de conocer la variación de las poblaciones de mosca blanca y establecer como las condiciones climáticas prevalentes, así como la presencia de hospederos, afectan su abundancia.

La instalación de las trampas en el campo se hizo forrando una "X", alternando las diferentes formas y alturas.

Los recuentos se hicieron cada 24 horas, de lunes a viernes (o a sábado cuando fué posible). La hora de recuento osciló entre las 8 y 9.30 A.M., anotando de cada trampa el número de B. tabaci capturado. Tan pronto se terminaba el recuento, se removían los insectos atrapados y con una brocha se agregaba más adherente.

Los datos climatológicos (1) de temperatura mínima, máxima y promedio, dada en °C, (2) de humedad relativa mínima, máxima y promedio, dada en %, (3) de velocidad del viento mínima, máxima y promedio, dada en km por hora, así como su dirección predominante, se tomaron diariamente del registro de la estación meteorológica, localizada a 800 m aproximadamente.

Resultados y Discusión

De los registros de captura realizado por las trampas a diferentes alturas (Tabla 5), se encontró que las trampas de forma cúbica capturaron 27. más que la de forma cilíndrica siendo la diferencia mayor, respecto a las de forma rectangular. La orientación de la trampa que tuvo solamente dos caras respecto a la dirección del viento, fué determinante para incrementar la captura. Las trampas que cubrieron con sus dos caras los puntos cardinales Norte-Sur, que es de donde provienen los vientos dominantes en esta localidad, incrementaron su captura en un 24 por ciento.

La prueba de independencia de χ^2 (Tabla 5), hecha con los totales de mosca blanca capturados por las trampas de forma

TABLA 5. PROCEDIMIENTO PARA EL CÁLCULO DE LA CARGA DE TRABAJO EN LAS ALTURAS DEL SUELO. CHILE, AÑO 1977.

ALTURA (1)	PUNTO DE TRABAJO				PORCENTAJE DE CARGA 1/
	1-1	1-2	1-3	1-4	
0.1	746	435	746	1062	26.15
0.2	157	172	210	420	10.34
0.3	205	275	275	9300	23.10
0.4	182	125	102	346	8.52
0.5	155	115	118	144	3.55
0.6	156	81	119	429	10.56
0.7	167	145	101	153	3.40
0.8	155	101	129	128	3.15
0.9	145	77	174	232	5.71
1.0	---	---	105	110	2.91
1.5	---	---	---	37	0.91
2.0	---	---	---	33	0.81
2.5	---	---	---	14	0.34
3.0	---	---	---	11	0.27
4.0	---	---	---	11	0.27

* CARGA DE TRABAJO EN EL SUELO DE LA ZONA DE TRABAJO.

1/ PORCENTAJE DE CARGA DE TRABAJO EN LA ZONA DE TRABAJO.

$$K_c^2 = 559.90 >> K_c^2 = 10.6$$

cúbica, indican que estadísticamente la altura de la trampa es importante también para aumentar la captura. El 60% se capturó de 0.1 a 0.3 m, el 38% de 0.4 a 1m y solamente el 2.6% de 1.5 a 4m de altura. La captura del 26% del total, a 0.1m de altura, sugiere que B. tabaci prefiere volar a niveles bajos, resultados que no concuerdan con los reportados por Amaya (1973). Aunque la captura de B. tabaci, fué relativamente mínima a niveles superiores de 1.5m, es factible que dichos insectos aprovechen corrientes de aire para movilizarse a mayores distancias. Lo anterior es algo que valdría la pena investigar.

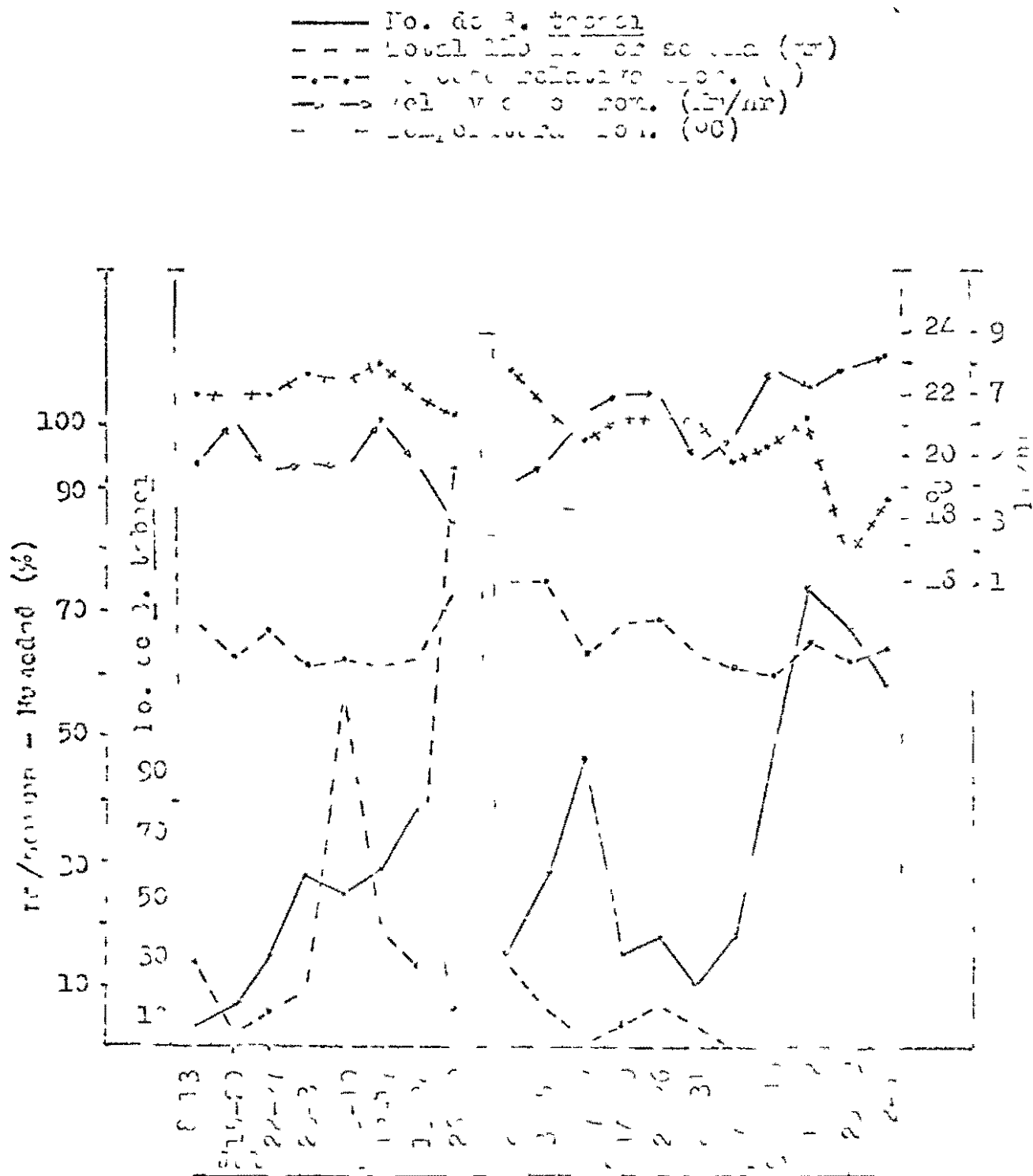
Las líneas de tendencia de promedio semanal ^{1/} de temperatura (°C), de humedad relativa (%), de velocidad del viento (km/hr) y del total de lluvia registrada; así como el promedio diario por trampa de moscas blancas, capturadas en trampas de forma cúbica instaladas a 0.1m de altura, se presentan en la Figura 3.

En Monjas se registraron del 8 de Julio al 6 de Diciembre, cuatro crestas mayores de poblaciones, coincidiendo dos con cada ciclo de cultivo de frijol. Las primeras dos se registraron del 29 de Julio al 3 de Agosto y del 19 al 24 del último mes, con promedios de captura diaria por trampa de 56 y 78 adultos, respectivamente. Las últimas dos crestas se presentaron del 7 al 12 de Octubre con 73 adultos y la última del 18 al 22 de Noviembre con 149 adultos por trampa. La primera cresta ocurrió cuando el cultivo (siembra de Junio-Agosto) estaba en ejote (vainas de unos 15 días de edad) y la segunda cuando el frijol estaba próximo a cosecharse. En la siembra de segunda (Septiembre-Diciembre), se registró la primera, cuando el cultivo tenía 27-31 días de edad y, la segunda una semana antes de la cosecha.

Según un estudio de reconocimiento de hospederas de B. tabaci y de B. fabae, realizado por el autor (1974), el frijol negro no es una hospedera preferida de B. tabaci, y en el valle de Monjas, la presencia de cultivos como el tabaco (Nicotiana glauca) y de tomate (Lycopersicon esculentum), así como la presencia de las coleas (escobillo (Cordia s.)) y del girasol silvestre (Helianthus sp) son hospederas más comunes durante el año, por lo que son más importantes para las poblaciones de B. tabaci.

^{1/} Los promedios de cada factor climático proceden de la suma del promedio diario, dividido entre el número de días.

Figura 3. Líneas de nivel de la velocidad de viento por
 sectores de viento (3 líneas) de ve-
 rificables en los datos prevalecientes. en las, y l.
 1974.



Respecto al efecto que tuvieron las condiciones climáticas, sobre las crestas y descensos de población, se observa (Fig. 3), que fueron semejantes en ambos casos, excepto por la -- precipitación. La lluvia fué generalmente más abundante durante las semanas que antecedieron a los descensos como durante éstos, en comparación con las dos semanas analizadas para cada cresta.

En las Tablas 6 y 7, se presentan las variables del factor lluvia que influyeron sobre las crestas y descensos de población.

Las variables del factor de lluvia más adversas para las poblaciones de B. tabaci, parecen ser la intensidad de la lluvia seguido de la duración de la misma. Lluvias registradas por espacio de 35 minutos a 8 horas por semana, tanto en la semana anterior al descenso de población como en ésta, a intensidad de 5 a 11 mm por hora, redujo la población de adultos desde el 41 hasta el 82 por ciento.

Las ventajas de éste tipo de estudios son: (1) Permiten -- orientar el uso de insecticidas a épocas oportunas, (2) Permiten reducir el nivel de poblaciones mediante el control de hospederos alternos, (3) Permiten usar calendarios de -- siembra como método de control integrado y, (4) Permiten en evaluaciones de germoplasma, programar la siembra y por consiguiente la toma de datos a épocas oportunas.

Selección y Plan de mejoramiento de Phaseolus vulgaris L. -- Por la preferencia para oviposición y Alimentación de B. tabaci.

Se han hecho esfuerzos tratando de encontrar fuentes de resistencia en frijol al "virus", agente causal de la enfermedad conocida como mosaico dorado, pero casi nada se ha hecho tratando de detectar fuentes de resistencia en Phaseolus vulgaris al vector Beauveria tabaci (Genn).

Husian et al mencionado por Painter (1951), indica que la resistencia a mosca blanca en algodón parece estar relacionada con diferencias en el pH de la sabia, pero que ésta varía de estación a estación y de un año a otro.

En base a un estudio de reconocimiento de hospederos de la

TABLE 6. VARIATION OF THE PERCENTAGE OF THE TOTAL AREA OF THE
 FISH HABITAT, SOON TO BE ESTABLISHED IN THE HABITAT OF THE
 YEAR 1974.

SEASON	PERCENT %	PERCENT (17)	PERCENT %	PERCENT %	PERCENT %
19-24 AUGUST		3.2	1	0-41	4.62
26-29 AUGUST	62.05	92.3	4	8-22	11.09
7-12 OCTOBER		0.7	1	0-18	2.33
14-19 OCTOBER	63.45	3.5	3	0-35	6.00
22-26 OCTOBER		6.9	2	1-10	5.79
28-31 OCTOBER*	41.67	3.9	1	0-39	6.00

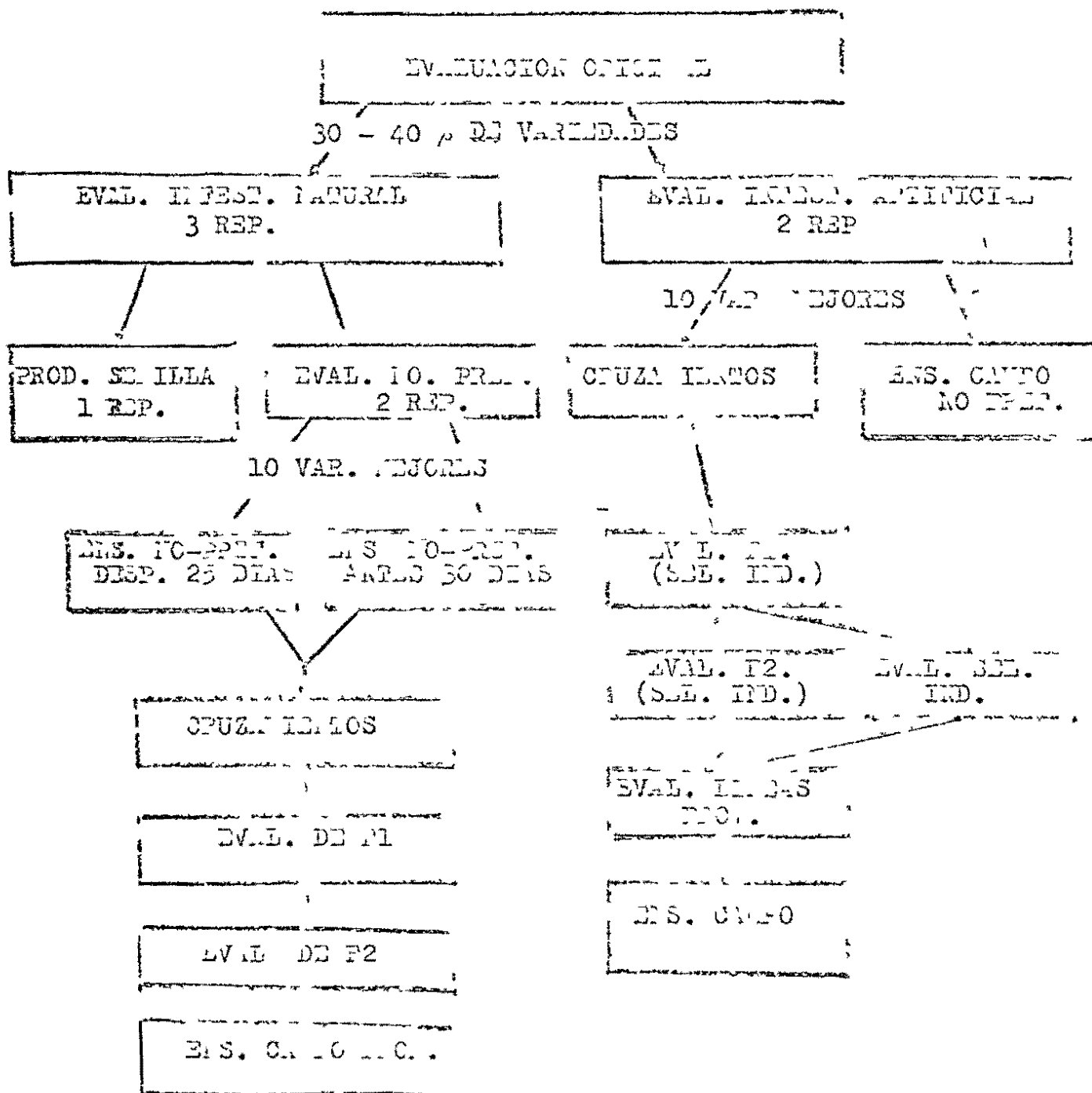
* SEE ALSO THE DATA OF THE PERCENTAGE OF THE
 FISH HABITAT.

TABLA 7. VARIABLES DEL TIEMPO DE LLOVIA QUE INTERVIENEN EN LAS CRESTAS DE POBLACION DE B. T. 1961, EN PUEBLOS, JUL. 1974.

SEMANA	AUMENTO %	LLOVIA (mm)	No. DIAS	No. HORAS	INTENSIDAD mm / Hr
22-27 JULIO		6.0	1	3-41'	1.62
22 JUL. 3 AG ⁺	46.43	8.7	2	1-15'	6.95
12-17 AGOSTO		19.2	2	5-00	3.84
19-24 AGOSTO ⁺	26.92	3.2	1	0-41'	4.68
30 SEP. - 5 OCT.		6.2	4	1-38'	3.79
7-12 OCTUBRE ⁺	38.71	0.7	1	0-18'	2.35
11-15 NOVIEMBRE	--	0.0	0	0-00	0.00
18-22 DICIEMBRE ⁺	38.25	0.0	0	0-00	0.00

+ SEMANAS DE CRESTAS DE POBLACION
1/ LLOVIA TOTAL.

DIAGRAMA DE SELECCION Y EJERCICIO DE Trascolus v. ... PA
RA AUMENTAR LA O-PREFERENCIA DE B. ...



mosca blanca y de la chicharrita verde Empoasca sp (Alonzo F. 1974a), y a un estudio de fluctuación de poblaciones también de estos dos insectos (Alonzo F. 1974b), se afirma que Phaseolus vulgaris no es una hospedera preferida de Bemisia.

El autor considera, por recuentos de ninfas efectuados en -- evaluaciones de gertoplasma, que es posible encontrar variedades con nivel alto, de no-preferencia para oviposición y -- quizá también para alimentación, con lo cual se reduciría -- considerablemente la frecuencia de plantas con mosaico dorado; así como el uso de insecticidas, a solamente épocas en -- que se presenten altas poblaciones.

También, puede ser posible elevar la no-preferencia para la oviposición y alimentación a un nivel más alto, mediante la cruce de los materiales seleccionados como promisorios según do de selecciones individuales en la progenie (F1 y F2).

La tarea sería mucho más fácil, si la no-preferencia para -- alimentarse y para ovipositar estuvieran gobernadas por características morfológicas o de estructura diferentes. Esto permitiría (mediante la recombinación génica inducida mediante cruzamientos, la concentración en algunos individuos, de un mayor potencial para no preferencia (varias características relacionadas).

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GENETIC METHODS OF INSECT CONTROL WITH
PARTICULAR REFERENCE TO MEXICAN BEAN BEETLE

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ok

INTRODUCTION:

The use of chemical pesticides to control pests of plants presents a great problem of toxic residues to personnel using the chemicals as well as to the consumer of the harvested plant products. In addition the use of pesticides causes development of resistant strains of insects, outbreaks of secondary pests resulting from the destruction of their natural enemies, and side effects on non-target organisms including man. Often the pest populations quickly return to higher level than before treatment. There is also direct danger to human health in the application of pesticides. Thus, the hazards from the use of pesticides to control pests of plants cannot be overemphasized.

In certain cases the chemical control of the pest is very difficult because the plants are harvested daily and chemical residues occur. Control methods such as genetic resistance in plants need to be exploited. Insect resistant plants do not harm beneficial insects, have very little effect in disturbing nature's balance between destructive insects and their natural enemies. They are compatible with other control methods (chemical, biological, or cultural). Even if the effective and safe insecticides become available in the distant future, a logical analogy would be "Why to fight a fire if a fire proof building is possible." Insect resistance involves minimum production costs as compared with the use of chemical pesticides to control insect-pests.

The total cost of research in the United States by Federal, State and Private Institutions on four insects of economic importance (Hessian Fly, Saw Fly, Alfalfa Aphid, and European Corn Borer) have been \$9.3 million. However, as a result of this effort the savings passed on to the consumer by way of lower food prices are estimated at \$310 million per year. The net pay off, thus have been \$300.00 per \$1.00 spent on host plant resistance. This is, of course, in addition to savings from eliminating chemicals and their residues, and future safeguard against insect-pests. There is, thus, pressing need to search for and maintain possible sources of genetic resistance. Locating resistant germ plasm to insects is the ideal way to safeguard plants against insect-pest losses and at the same time preventing pollution of the environment and health hazards to human beings.

GENETICS OF INSECT RESISTANCE

The pioneer work on insect resistance was done in horticultural crops on woolly apple aphid (13) and grape phylloxera in 1854 (9). However, as of now horticultural crops have fallen farther behind in insect resistance studies than field and forage crops.

A significant progress has been made in developing varieties resistant to insects in cereal and forage crops and varieties resistant to Hessian fly in case of wheat, and spotted alfalfa aphid in case of alfalfa are extensively grown (12, 15, 25). Whereas there are only few vegetable varieties available which are resistant to insects. Similarly there is need of extensive search for genetic resistance of plants to insects in high protein leguminous crops of economic importance. There are many references in literature on insect resistance in vegetable crops and various reviews have been published (1, 2, 17, 22, 23, 24, 25, 26) but only a few thorough studies have been made to

screen for insect resistance and to determine the nature and genetics of insect resistance Benepal and Hall (3, 4, 5, 6, 7, 8) have made several studies to determine the nature and genetics of insect resistance in vegetable crops Stoner (26) in a review writes that resistance to 37 insect species in 25 vegetable crops have been reported but most of these studies are based on adapted varieties and lines Hardly any effort has been made to follow search in depth and to maintain germ plasm for insect resistance There is a basic need to locate genetic resistance in plants that could be further utilized in developing resistant varieties and help reduce health hazards due to pesticides (18, 23, 26) After the available germ plasm is screened and resistance has been found, the next step should be to determine the genetic basis or inheritance of resistance The chemical basis of resistance may also be very useful in screening for insect resistance and in breeding programs

Insect resistance is a complex of many factors and is affected by various environmental factors such as temperature, humidity, fertility of soil etc Besides, biological forms of insects add to the complexity of the problem However, the fact that plants differ in their resistance to insects was recognized as early as 1778 (11) As defined by Painter (19), the resistance of plants to insects is a relative amount of heritable quality possessed by the plant which influences the ultimate degree of damage done by an insect Plants sometime may escape infestation or insect damage due to various reasons Thus it does not necessarily mean that in a susceptible variety a plant not infested is resistant It cannot be overemphasized that studies of its progeny will confirm the results Physiological, and structural characters are related to resistance to insects Therefore, a

resistance is a function of the genes which determine the hereditary characters of plants. It is, of course, a natural development by evolution which may be further exploited by the plant breeder.

A knowledge of the factors contributing to immunity or resistance and the conditions which influence these factors are valuable but not essential (2, 21, 24) since varieties of wheat and alfalfa have been evolved without any definite knowledge of basis of resistance. At the same time the need to pay a special attention to the basis of resistance in crop plants cannot be overemphasized. The financial limitations rather than the need, has been the cause of not having well balanced insect resistance programs. The real progress in this area can be expected only with a combined concerted input from various disciplines such as entomology, plant pathology, plant breeding, genetics, plant biochemistry and plant physiology and organic chemistry. A knowledge of genetic basis is very essential for all genetic investigations and extremely valuable to a plant breeder. The genetic analysis can help in differentiating resistance mechanisms occurring simultaneously in a single strain. This can in turn lead to a knowledge of the cause(s) of resistance. Thus, a relationship of resistance can be established with physiological and biochemical characters of crop plants.

The resistance of plants to disease and insects has been developed by natural selection and evolution since the resistant plants could have been exposed to a given insect or disease for a long time. Of course, the opposite could be true in case the host plant lacks necessary resistance to the same pest, and may be resistant to other insects. No doubt there are problems in which insects resistance offers an ample solution and in many cases it is necessary to select plants with fair degree of resistance combined

with other desirable characters rather than to have the highest resistance. Immune varieties are very rare. Resistance could be immunity or as low as a little more than low susceptibility. Level of resistance could be of great economic value from the insecticidal control (10). The host plant resistance is not a control method, however, insect resistant varieties do offer a great potential in effective insect control and must be included in breeding programs.

Breeding for insect resistance is not basically different from breeding for various other desirable traits in plants. Many interrelationships may be responsible for host plant resistance. It is desirable to combine other desirable plant characteristics when breeding for insect resistance from a plant lacking desirable characteristics for other traits.

Quality can be transferred to another commercially acceptable variety by crossing, selection, crossing followed by selection, and

utilized in developing varieties resistant to insects.

It is a waste of time and effort to attempt to breed for insect resistance if resistance can be found in some variety already present in the country. Therefore, one must look for new sources. The next step should be to mass screen plant varieties from various countries. However, it is recognized that the introduction of varieties may have adaptation limitations to a new locality. Numerous instances of insect resistance being obtained from various sources.

Selection is the simplest method of obtaining plants resistant to insects. It can only be practiced if there are adequate insect infestations.

plants Artificial insect infestations can facilitate the progress of selecting resistant plants However, this method is limited by the extent of genetic resistance carried by the heterozygous variety or plant introduction

Crossing followed by selection has yielded excellent results in the development of resistance varieties The objective of hybridization is to combine in a single variety the desirable characters of two or more lines, varieties or species The pedigree, bulk and backcross methods have been used successfully in developing resistant varieties. Multiple resistance to insects could also be accomplished by recurrent phenotype selection. Synthetic varieties of alfalfa with resistance to more than one insect-pest have been developed In breeding for resistance to insect-pests efforts should be made to locate and combine as many sources of resistance as possible to have a broad genetic basis against biotypes Of course, induced mutation is the only available choice if no genes for resistance to a specific insect-pest are available in a crop plant

GENERAL ORGANIZATION OF THE PROGRAM AT VIRGINIA STATE COLLEGE

The research program, Plant Germ Plasm Resources For Improved Quality and Food Safety, was approved by the Cooperative State Research Service, U S Department of Agriculture in 1972 and I have been directing this program since its inception The following research projects have so far been developed

- 1 Genetic Means of Control of Insect-Pests Through Resistant Plant Germ Plasm Resources
- 2 Genetic Means of Control of Diseases Through Resistant Plant Germ Plasm Resources
- 3 Biochemical Components Especially Protein and Quality of Protein of Selected Plant Germ Plasm Resources

The primary objectives of the research project, Genetic Means of Control of Insect-Pests Through Resistant Plant Germ Plasm Resources, are as follows

To locate sources of resistance in plants especially vegetable crops, and leguminous crops of economic importance

To determine the components of resistance involved

To maintain and share resistant germ plasm with other interested scientists

A team approach to the problem of controlling insect-pests and diseases by resistant varieties and improvement of nutritional value of selected crop plants is being established. The present research staff at scientists level on the research program is as follows

Program Leader

Entomologist

Plant Pathologist

Plant Biochemist

Plant Breeder

Post-Doctoral Fellow (Biochemistry/Physiology of Insect-Disease Resistance)

Post-Doctoral Fellow (Organic Chemistry)

Post-Doctoral Fellow (Ultrastructure-Insect/Disease Resistance)

The following facilities and major equipment will also be available: Scanning Electron Microscope, Amino Acid Analyzer with Integrator, GC-Mass Spectrophotometer, Gas Chromatographs, Grain Quality Analyzer (for moisture, ash, protein and oil determinations etc), U V Visible Spectrophotometer with Kinetic System, Infrared Spectrophotometer, Ultracentrifuges, Electron Area Meter, Climate Control Greenhouse, Permanent Germ Plasm Storage, Computer IBM-370-25 etc

PROGRESS MADE

Bean Phaseolus vulgaris L and cabbage Brassica oleracea var capitata L were initially included in the study, while soybean Glycine max L

(Merrill) was included during the year 1974. The main insects under study are Mexican bean beetle in case of bean and soybean and cabbage looper and imported cabbage worm in case of cabbage. I will, however, restrict my discussion primarily to the control of Mexican bean beetle by resistant varieties.

An eight feet observational row of each of the cultivars was planted for study under field conditions of natural insect populations. A comprehensive record of botanical characteristics and resistance of each of the cultivars to Mexican bean beetle was made as shown in 'Key' for Entomological and Botanical characteristics on the following pages. In addition, disease resistance ratings were also taken as given in 'Key' (items 39-44). It is also planned to study the following biochemical quality characteristics of selected lines: moisture, ash, total carbohydrates, dietary fiber, oil, protein, and amino acid profile.

Some 5,652 cultivars of beans were acquired through the courtesy of Plant Introduction Station, U.S. Department of Agriculture, Washington State University, Pullman, Washington and various seed companies. During the year 1973, out of 2,340 collections screened, 117 showed a foliage damage of 0-10% under field conditions of natural insect populations (Table 1). Whereas 4,140 cultivars were screened in 1974 out of which 144 showed a damage rating of 1 (0-10% foliage damage). The overall Mexican bean beetle damage was approximately 20% greater during 1974 than in 1973. 1,338 cultivars were screened during the year 1975 and 37 showed a foliage damage of less than 10%. Thus about 5% of the total germ plasm screened have been selected for further detailed study. A no choice feeding preference test under controlled conditions is being conducted on the selected cultivars. A dual choice test on all selected

KEY

ENTOMOLOGICAL AND BOTANICAL CHARACTERISTICS

BEANS

1974

1. SERIAL NUMBER

2. PLANT INTRODUCTION (P.I.) OR VARIETY: CC = COMMERCIAL CODE UNK = UNKNOWN

3. COUNTRY OF ORIGIN*

AFG = AFGAHANISTAN
AFR = AFRICA
ARA = ARABIA
ARG = ARGENTINA
ASM = ASIA MINOR
ARZ = ARIZONA
AUS = AUSTRALIA
BCO = BELGIUM CONGO
BEL = BELIGIUM
BOL = BOLIVIA
BRC = BRITICH CAMER
BRZ = BRAZIL
BUG = BULGARIA
BUM = BURMA
CAL = CALIFORNIA
CAM = CENTRAL AMERICA
CAN = CANADA
CHE = CHILE
CHN = CHINA
CLS = CHANNEL ISLAND
COL = COLUMBIA
CRA = COSTA RICA
CSR = CZECHOSLOVAK
SOCIALIST REP.
CUB = CUBA
DEN = DENMARK
ECU = ECUADOR
EAF = EAST AFRICA
EGY = EGYPT
ELS = EL SALVADOR

ENG = ENGLAND
EPK = EAST PAKISTAN
ETH = ETHIOPIA
FRC = FRENCH CAMER.
FRN = FRANCE
GEO = GEORGIA
GER = GERMANY
GRE = GREECE
GUA = GUATAMULA
HAT = HAITI
HON = HONDURAS
HUN = HUNGARY
IND = INDIA
IRN = IRAN
ITY = ITALY
JAP = JAPAN
KEA = KOREA
KEN = KENYA
LEB = LEBANON
MEX = MEXICO
MNE = MAINE
NDK = NORTH DAKOTA
NEP = NEPAL
NET = NETHERLANDS
NHP = NEW HAMPSHIRE
NIC = NICARAGUA
NMX = NEW MEXICO
NOR = NORWAY
NYK = NEW YORK
PAL = PALESTINE

PAR = PARAGUAY
PEU = PERU
PIS = PHILIPINE ISLANDS
POL = POLAND
POR = PORTUGAL
PRI = PUERTO RICO
RUU = RUANDA-URUNDA
SAF = SOUTH AFRICA
SCT = SCOTLAND
SPN = SPAIN
SRH = SOUTH RHODESIA
SWD = SWEDEN
SWZ = SWITZERLAND
SYR = SYRIA
THA = THAILAND
TAN = TANZANIA
TUR = TURKEY
UGD = UGANDA
UKR = UKRAINE
USA = UNITED ST. OF
AMERICA
USR = UN. OF SOV. SOC.
REPUBLIC
UTK = UNITED K. OF
G.B. & N
VEN = VENEZULA
WGR = WEST GERMANY
WPK = WEST PAKISTAN
YUG = YUGOSLAVIA

*BLANK REPRESENTS COMMERCIAL VARIETIES.

KEY

4. MATURITY: E = EARLY, M = MEDIUM AND L = LATE
5. HABIT OF GROWTH: B = BUSH, R = RUNNER & P = POLE
6. VIGOR: 1 = LEAST TO 5 = MOST
7. UNIFORMITY: 1 = LEAST TO 5 = MOST
8. PLANT HEIGHT (CMS.)
9. RACEME LENGTH: 1 = 4, 2 = 4 TO 8, 3 = 8 TO 12, 4 = 12 TO 16,
AND 5 = 16 CMS.
10. FOLIAGE. 1 = LEAST TO 5 = MOST
11. LEAF SIZE: 1 = 2.5, 2 = 2.5 TO 5, 3 = 5 TO 7.5, 4 = 7.5 TO 10,
AND 5 = 10 CMS.
12. PETIOLE LENGTH: 1 = 5, 2 = 5 TO 7.5, 3 = 7.5 TO 10, 4 = 10 TO 12.5
AND 5 = 12.5 CMS.
13. INTERNODE LENGTH: 1 = 2.5, 2 = 2.5 TO 5, 3 = 5 TO 10, 4 = 10
TO 15, AND 5 = 15 CMS.
14. BRANCHING: 1 = LEAST TO 5 = MOST
15. FLOWER COLOR: L = LAVENDER, W = WHITE, Y = YELLOW, PR = PURPLE
AND PI = PINK
16. POD SET: 1 = LEAST TO 5 = MOST
17. POD LENGTH: 1 = 5, 2 = 5 TO 7.5, 3 = 7.5 TO 10, 4 = 10 TO 12.5
AND 5 = 12.5 CMS.
18. POD WIDTH: 1 = 0.5, 2 = 0.5 TO 1, 3 = 1 TO 1.5, 4 = 1.5 TO 2,
AND 5 = 2 CMS.
19. POD BEAK: 1 = 0.5, 2 = 0.5 TO 1, 3 = 1 TO 1.5, 4 = 1.5 TO 2,
AND 5 = 2 CMS.
20. POD CONSTRICTION: 1 = LEAST TO 5 = MOST
21. POD COLOR: 1 = LIGHT GREEN, 2 = GREEN, 3 = DARK GREEN,
4 = YELLOW, 5 = SPLASH, AND 6 = PURPLE
22. POD SHAPE (CROSS SECTION): 1 = ROUND TO 5 = FLAT
23. POD CURVATURE 1 = STRAIGHT TO 5 = CURVED
24. POD STRINGINESS 1 = STRINGLESS AND 2 = STRING
25. POD FIBER. 1 = LEAST TO 5 = MOST

KEY

-11-

26. SEED SIZE: 1 = LEAST TO 5 = LARGE

27. SEED SHAPE: T = TRUNCATE, P = PLUMP, Ov = OVAL, F = FLAT,
AND Ob = OBLONG

28. SEED COLOR: A = FLECKED, B = MOTTLED, C = STREAKED, D = BROWN,
E = WHITE, F = TAN, G = SPLASH, H = RED, I = PURPLE,
J = BLACK, K = GREEN, AND L = YELLOW.

29. MEXICAN BEAN BEETLE (NATURAL INFESTATION): 1 = LEAST TO 5 = MOST

30. LEAF HOPPER (NATURAL INFESTATION): 1 = LEAST TO 5 = MOST

31. PREVALENCE OF INSECT DAMAGE: NUMBER OF PLANTS PER CULTIVAR WITH
MEXICAN BEAN BEETLE INFESTATION/
NUMBER OF PLANTS PER CULTIVAR.

32. INTENSITY OF INSECT DAMAGE ON LEAVES: NUMBER OF DAMAGED LEAVES
(LEAVES WITH A MINIMUM OF
ONE CENTIMETER SQUARE OF
TISSUE DAMAGED) 50 RANDOMLY
SELECTED LEAVES PER CULTIVAR

33. PODS DAMAGED: 1 = 0 TO 10%, 2 = 10 TO 20%, 3 = 20 TO 30%,
4 = 30 TO 40%, 5 = 40 TO 50%, 6 = 50 TO 60%,
7 = 60 TO 70%, 8 = 70 TO 80%, 9 = 80 TO 90%,
AND 10 = 90 TO 100%.

34. LEAF HOPPER DAMAGE (VISUAL ESTIMATION OF HOPPER BURN ON LEAVES).
SEE SCALE 33,

35. MEXICAN BEAN BEETLE DAMAGE (VISUAL ESTIMATION OF LEAF AREA
DAMAGED BY MEXICAN BEAN BEETLE
ADULTS AND LARVAE) SEE SCALE 33.

36. COMBINED DAMAGE RATING: THE ADDITION OF RATINGS FOR LEAF HOPPER
AND MEXICAN BEAN BEETLE).

37. OVERALL RATING LEAF HOPPER: H.S. = HIGHLY SUSCEPTIBLE,
S = SUSCEPTIBLE
M.R. = MODERATELY RESISTANT
R. = RESISTANT
H.R. = HIGHLY RESISTANT

38. OVERALL RATING = MEXICAN BEAN BEETLE. SEE SCALE FOR 37.

KEY

DISEASE RESISTANCE:

39. ROOT ROT FUSARIUM SOLANI F.
PHASEOLI AND RHIZOCTONIA
SOLANI

1 = HEALTHY (NO DISEASE)
2 = SLIGHTLY DISEASED
3 = MODERATELY DISEASED
4 = SEVERELY DISEASED
5 = PLANT DEAD

40. ANTHRACNOSE COLLECTOTRICHUM
LINDEMUTHIANUM.

1 = HEALTHY PODS (NO DISEASE)
2 = SLIGHTLY DISEASED
3 = MODERATELY DISEASED
4 = SEVERELY DISEASED
5 = PODS ROTTING

41. BACTERIAL BLIGHT
XANTHOMONAS PHASEOLI

1 = NO DISEASE SYMPTOMS
2 = FEW LEAF SPOTS
3 = MODERATE NUMBER OF LEAF SPOTS
4 = NUMEROUS LEAF SPOTS
5 = SEVERE LEAF SPOTTING

42. BEAN MOSAIC VIRUS:

1 = HEALTHY LEAVES (NO SYMPTOM)
2 = SLIGHT DISEASE SYMPTOMS
3 = MODERATE DISEASE SYMPTOMS
4 = NUMEROUS LEAVES SHOWING -
DISEASE SYMPTOMS
5 = PLANT WITH SEVERE DISEASE

43. BLACK ROT
XANTHOMONAS CAMPESTRIS

1 ----- 5

44. BLACK LEG
PHOMA LINGAM

1 ----- 5

TABLE 1.

TOTAL NUMBER OF BEAN CULTIVARS
ACQUIRED, SCREENED AND SELECTED

	1973	1974	1975
CULTIVARS SCREENED	2,340	4,140	1,000
CULTIVARS SELECTED FOR FURTHER STUDY	<u>177</u>	<u>144</u>	<u>37</u>
TOTAL NUMBER OF CULTIVARS ACQUIRED	5,624		

cultivars will also be made. A susceptible variety will be included along with the selected resistant cultivar. It is possible that a combination of susceptible with resistant or moderately resistant could be used as a cultural control method especially in kitchen gardens or on small farms. A partial list of comparatively resistant germ plasm of beans to Mexican bean beetle screened under field conditions during 1973, 1974 and/or 1975 is given in Table 2. A heavy infestation of Mexican bean beetles existed during the years 1974 & 1975. The same fields were used during each of the years for bean plantings. Sixteen cultivars showed less than 10% foliage damage during two or three years of study.

A preliminary study was done to determine the non-preference of Mexican bean beetles to 31 selected cultivars with a damage rating of 1 (0-10% foliage damage). A randomized complete block design with ten replications was used. Each treatment consisted of a leaf from 31 genotypes screened during 1975. The first mature leaf from the top of the full grown plant was used. Each leaf was placed in a transparent plastic cup 2"x1" size containing distilled water. This cup was placed in another 5"x4" clear plastic container with a perforated lid. One adult Mexican bean beetle was released on each leaf. The leaf consumption was determined with the help of a grid after 24 hours of Mexican bean beetle feeding. Leaf damage ($\text{cm}^2/24$ hours) on each cultivar is given in Table 3.

There was a significant difference in Mexican bean beetle feeding preference for various selected cultivars. The leaf consumption varied from 0-7 $\text{cm}^2/24$ hours. According to Duncan's Multiple Range Test the first 13 lines did not differ significantly, whereas the last three cultivars were most preferred. A feeding of 2 cm^2 of foliage was considered low. At least

TABLE 2.

COMPARATIVELY RESISTANT GERM PLASM OF BEANS TO MEXICAN BEAN
BEETLE SCREENED UNDER FIELD CONDITIONS DURING 1973, 1974 AND/
OR 1975 (PARTIAL LIST)

CULTIVAR	DAMAGE RATING ¹	CULTIVAR	DAMAGE RATING
P.I. 151392	1	P.I. 290996	1
P.I. 152211	1	P.I. 299382	1
P.I. 174910	1	P.I. 308900	1
P.I. 183343	1	P.I. 309695*	1
P.I. 195350	1	P.I. 309706	1
P.I. 201331*	1	P.I. 309879	1
P.I. 201380	1	P.I. 310778	1
P.I. 206223*	1	P.I. 311824	1
P.I. 206979*	1	P.I. 311862	1
P.I. 207212	1	P.I. 319608	1
P.I. 209489	1	P.I. 324600*	1
P.I. 209201*	1	P.I. 326347*	1
P.I. 228358	1	P.I. 338972*	1
P.I. 250166*	1	P.I. 339423*	1
P.I. 271501	1	P.I. 352753*	1
P.I. 281595*	1	P.I. 368061*	1
P.I. 281999*	1	CC00 37*	1
		CC00 75*	1

¹VISUAL ESTIMATION OF TOTAL LEAF DAMAGE ON A SCALE OF 1-10
AS FOLLOWS 1=0-10, 2=11-20, 3=21-30, 4=31-40, 5=41-50,
6=51-60, 7=61-70, 8=71-80, 9=81-90, 10=91-100%.

*CULTIVARS SHOWING LESS THAN 10% FOLIAGE DAMAGE DURING TWO OR
THREE YEARS OF STUDY.

TABLE 3.

ADULT MEXICAN BEAN BEETLE LEAF FEEDING PREFERENCE
ON 31 BEAN CULTIVARS¹ (NO CHOICE BASIS)

S. No.	CULTIVAR	LEAF DAMAGE CMS ² /24 HRS.	S. No.	CULTIVAR	LEAF DAMAGE CMS ² /24 HRS.
1	CC0078	0.0	17	207211	2.1
2	CC0037	0.4	18	207186	2.1
3	306151	0.6	19	352753	2.3
4	250166	0.9	20	306153	2.3
5	CC0075	1.0	21	310897	2.3
6	311862	1.4	22	313608	2.4
7	311824	1.5	23	310695	2.4
8	299389	1.5	24	299386	2.6
9	313882	1.5	25	309698	3.4
10	299383	1.7	26	309879	3.4
11	CC0036	1.7	27	361505	4.4
12	339491	1.8	28	313709	4.8
13	281999	1.8	29	361171	5.9
14	326347	1.9	30	247699	6.4
15	345577	2.0	31	361504	7.0
16	282108	2.1			

¹ALL CULTIVARS HAD A LEAF DAMAGE RATING OF 1(0 0% LEAF DAMAGE)
UNDER FIELD CONDITIONS.

the first 13 lines appear to be resistant. The experiment will be repeated with 4 Mexican bean beetles per leaf rather than one and leaf consumption will be determined with the help of an electronic area meter.

Nine of the 53 cultivars of soybean screened during 1974 had a damage rating of 1 (0-10% foliage damage) and appear to be comparatively resistant to Mexican bean beetle (Table 4). The Mexican beetle leaf damage was comparatively less on soybean as compared with on beans.

An effort was also made to determine the effect of Mexican beetle foliage damage on productivity and quality of bean seed and the results are shown in Tables 5 & 6. Simulated insect damage conditions were created by removing 5, 13, 26, 32, and 39% of plant foliage. Other treatments were natural Mexican bean beetle infestation, release of 3 Mexican bean beetles per plant, and 6 Mexican bean beetles per plant. The experiments were conducted under wire cages and a randomized block design with 4 replications was used. The experiments were repeated for 3 years under field conditions. The results indicate that 7-10% of leaf damage does not affect the productivity significantly, whereas reduction in plant productivity due to natural insect-infestation (11-20% foliage damage) was 12%, and 3 Mexican beetles (31-40% foliage damage) and 6 Mexican beetle (71-80% foliage damage) reduced 28 and 62% of plant productivity, respectively as compared with the control (no insect damage). There seems to be a direct relationship between the foliage damage and reduction in yield. In addition, higher infestations of Mexican beetle seem to reduce protein and oil content of bean seed. Thus a visual leaf damage rating of 1 (less than 10% foliage damage) can be safely used to characterize a plant resistant since the productivity and quality of the plant is not affected significantly at this level.

TABLE 4. MEXICAN BEAN BEETLE LEAF DAMAGE ON 53 CULTIVARS OF SOYBEANS SCREENED UNDER FIELD CONDITONS DURING 1975

SOYBEAN CULTIVARS	DAMAGE ¹ RATING	SOYBEAN CULTIVARS	DAMAGE RATING
MAMMOTH YELLOW	1	171443	2
HILL	1	196177	2
D217599	1	201421	2
170887	1	201428	2
171442	1	227158	2
200461	1	D703115	2
227687	1		
229358	1	ESSEX	3
BRAGG	2	PALMETTO	3
EDNEST	2	PINEDILL	3
LAREDO	2	RANSOM	3
LEE	2	ROANOKE	3
MACK	2	TANNER	3
TOKYO	2	62204	3
WOOD YELLOW	2	84874	3
83942	2	157413	3
85465	2	157451	3
87542	2	170888	3
89061	2	170895	3
90481	2	201422	3
95035	2	227557	3
96089	2	229350	3
157440	2	D703185	3
170890	2		
171437	2	ARBSOY	4
		85342	4
		175193	4
		181547	4

¹VISUAL ESTIMATION ON A SCALE OF 1 TO 10 AS FOLLOWS: 1=0 TO 10, 2=10.01 TO 20, 3=20.01 TO 30, 4= 30.01 TO 40, 5= 40.01 TO 50, 6=50.01 TO 60, 7= 60.01 TO 70, 8=70.01 TO 80, 9= 80.01 TO 90, AND 10=90.01 TO 100%.

EFFECT OF MEXICAN BEAN BEETLE DAMAGE ON PRODUCTIVITY OF BEAN SEED

	YIELD/PLANT IN G.							
	YEAR I		YEAR II		YEAR III		AVERAGE FOR 3 YEARS	
	YIELD	%DECREASE	YIELD	%DECREASE	YIELD	%DECREASE	YIELD	%DECREASE
% OF LEAVES REMOVED								
CONTROL	92.4	----	21.5	----	18.6	----	44.2	----
6.5	91.5	1.2	19.7	8.4	13.6	26.9	41.6	5.9
13.0	87.4	5.4	14.9	30.7	11.2	39.8	37.8	14.5
19.5	80.3	13.1	13.8	35.8	10.8	41.9	34.9	21.0
26.0	71.3	22.8	13.6	36.7	9.6	48.4	31.5	28.7
32.5	68.4	26.0	12.6	41.4	10.3	44.6	30.4	31.2
39.0	65.3	29.3	12.3	42.8	7.0	62.4	28.2	36.2
INSECT INFESTATION								
NATURAL INFESTATION	83.3	9.8	18.9	12.1	14.1	24.2	38.8	12.2
3 MBB/PLANT	76.9	16.8	10.4	51.6	7.5	59.7	31.6	28.5
6 MBB/PLANT	----	----	9.1	57.7	6.4	65.6	7.7	61.7
LSD AT .05	5.7	----	2.1	----	1.9	----	3.3	----

EFFECT OF MEXICAN BEAN BEETLE DAMAGE ON QUALITY OF BEAN SEED

	PROTEIN %				OIL %			
	YEAR I	YEAR II	YEAR III	AVERAGE	YEAR I	YEAR II	YEAR III	AVERAGE
<u>% OF LEAVES REMOVED</u>								
CONTROL	34.2	35.0	35.7	35.0	22.4	21.3	21.4	21.7
6.5	34.2	35.1	35.6	35.0	22.2	21.1	20.9	21.4
13.0	33.6	34.8	35.2	34.5	22.7	20.6	20.9	21.4
19.5	33.8	34.6	34.4	34.3	22.7	20.7	20.9	21.4
26.0	33.4	34.2	34.5	33.9	22.4	20.7	20.9	21.3
32.5	33.1	33.6	34.1	33.6	22.7	20.4	20.7	21.2
39.0	32.6	33.7	34.0	33.4	22.5	20.1	20.6	21.0
<u>INSECT INFESTATION</u>								
NATURAL INFESTATION	32.9	34.4	34.6	34.0	22.1	21.2	19.1	20.8
3 MBB/PLANT	33.0	33.9	33.4	33.4	21.2	20.1	19.2	20.1
6 MBB/PLANT	----	33.0	33.1	33.1	----	19.8	18.8	19.3

After a thorough screening of the available germ plasm, studies on the nature and genetics of resistance will be conducted. Induced mutants, which has the possibility of adding resistance without giving up the desirable characteristics of a commercial variety, will also be studied. Any resistant germ plasm located will be shared with other interested scientists.

One of the main problems in a well balanced resistance program involving scientists from various disciplines could be of a human nature. The team of scientist must not only be well qualified in their respective areas but also understand, appreciate, and cooperate in all phases of the work. They should be cognizant of the difficulties and problems in other areas than their own. A team spirit and full cooperation among the research scientists working as a team is very essential element in the success of a research program.

There is a problem of handling huge data on botanical, entomological, pathological, and nutritional characteristics on large number of cultivars being screened. As of this year all field data are recorded on preprinted sheets. These sheets are directly used for punch cards. When the data for the three years are punched on computer cards correlations among various characteristics and resistance to insect-pests and diseases among various cultivars screened will be determined.

The Plant Introduction Accessions in certain cases are quite heterozygous. Seed from different plants in such cases is harvested separately. Because of low supply of seeds, hand plantings are done which take considerable time. May be a counted number of seeds could be put on a seed tape during off season prior to planting and these seed tapes could be laid out in field at planting time. Insect infestations have not been a problem under field conditions. An early planting of an alternate row of a susceptible variety can help

increase insect infestations. Occasional replacement of laboratory populations of insects with those in the field will be desirable

SUMMARY

There will be an intensified search for host plant resistance to insect-pests and diseases in the future and efforts will be made to combine the desired genes for nutritional value and insect and disease resistance to more than one insect or disease in a single variety. The development of better and less time consuming methods of screening germ plasm will be needed. The chemical basis of resistance and knowledge about the factor(s) that govern host specificity in insect pests may also be very useful in controlling insect-pests.

Virginia State College is trying to establish a team approach to the problem of controlling insect-pests by genetic means. Bean Phaseolus vulgaris L. and cabbage Brassica oleracea var capitata L. germ plasm is being thoroughly screened for insect-pests and diseases, and nutritional quality. The bean germ plasm seems to have potential sources of resistance to Mexican bean beetle. Further detailed studies under controlled conditions are needed to select the most valuable germ plasm.

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THE CONTROL OF BEAN INSECTS IN LATIN AMERICA

Dr. Léonce Bonnefil

Oh

This paper is intended to be a brief overview of the bean insects problem in Latin America with an emphasis on the necessity to strengthen cultural and biological controls. The protective role of these strategies has indeed gained considerable importance in these last few years. It is everybody's knowledge that because of the adverse effects on non-target organisms and their insidious effect on the total ecosystem, many pesticides are now being phased out or are being considered for phasing out in the countries where they are manufactured.

Although these compounds continue to be exported to other countries where they are not manufactured, these will eventually embark on environmental studies which will tend to limit the use of agricultural chemicals dangerous to man, plants and wildlife and enhance natural controls.

Chemical control of bean insect pests

It may be considered fortunate that bean production in Latin America has never been overly dependent on pesticides. This is not because bean plantings cannot be critically damaged by insects, but because bean culture in that region is predominantly a small domestic enterprise of low financial return which cannot very well pay for the cost of chemical control. Nevertheless in the countries where beans are produced on a commercial scale, insecticides have been used and presumably will continue to be used.

As for most crops, among the pesticides used, there is a definite shift in favor of non-persistent, fast-acting, narrow-spectrum insecticides (2). The latest recommendations of the United States Department of Agriculture (5) and of the University of Florida (3) for the major bean pests are listed below.

The following compounds are suggested to control aphids (Aphis) Diazinon, Demeton (Systox), Dimethoate

(Cygon, De-Fend), Parathion, Mevinphos (Phosdrin), Mal[~]thion, Demeton and Dimethoate are systemic, the rest act on contact. All of them (with the exception of Malathion) are highly toxic and require that they be applied only by trained operators.

Against the Mexican Bean Beetle (Epilachnia) are suggested Diazinon, Malathion, Parathion, Carbaryl (Sevin), Carbophenothion, Dimethoate, Methoxychlor, Disulfoton, Rotenone. Disulfoton and Parathion should not be used in home gardens and should be applied only by trained applicators. Treated plants should not fed to livestock. Carbophenotnion should not be used more than twice per season. Methoxychlor is the only organo-chlorinated compound in the group; unlike most products of its category, it shows little to no tendency to accumulate in the body fat of animals and is generally not toxic to crop plants.

The Bean Pod Weevil (Apion spp.) is best controlled

by repeated applications of fast-acting insecticides such as Parathion or Carbaryl but these applications should be made only when the insect can actually be seen.

The treatment recommended against leafhoppers (Empoasca spp.) makes use of systemics like Dimethoate or Disyston (Disulfoton), but Carbaryl (Sevin), Methoxychlor, Parathion or Malathion can also be used in foliar application.

The Banded Cucumber Beetle (Diabrotica), Leaf Beetles (Ceratoma, Andrector) are controlled with Carbaryl (Sevin), Diazinon, Malathion, Methoxychlor. There is^a tendency to be overly alarmed by the spectacular damage inflicted by the adults of these beetles₁ to the bean foliage although the damage caused by their larvae to the roots is often even more important.

Whiteflies (Aleurotrixus), vectors of the golden yellow mosaic, are prone to develop resistance when the

insecticides applications against the other bean pests are exceedingly frequent. They can then take up the status of primary pests.

Cultural and biological controls of bean insect pests

Generally, the insecticide recommendations have not varied significantly in the last few years largely because none of the insecticides used in the control of the common bean insect pests has been phased out or limited in its use. This in itself is a favorable situation but the end results would be just as significant, less costly and certainly easier on the environment if the application was made minimal. In particular, spraying should be properly timed. As a rule, it is best to wait until the population of a certain pest has developed to the point that its impact on the plant is economically significant - has reached the Economic Injury Level. A good example is Diabrotica which can

remove a large portion of the leaf area without serious effect on the production of the bean plant. Conversely, a high population of the leafhopper Empoasca at the early stages of the growth of the bean plant, will cause stunting, leaf cupping, yellowing and a grain production next to null.

*Kraemer on lowlands
phacelia on high lands*

In every case, sound cultural practices within or around the plantation will help cut down the building-up of large invading populations or the carry-over of great numbers of adults from one planting to the next.

Plowing in addition to destroying weeds, volunteer bean plants and wild hosts will also contribute to the eradication of rootworms, wireworms, cutworms, exposing them to farm animals and natural enemies.

Formal biological control of bean insect pests has received relatively little attention in Latin America in spite of the fact that there have been identified predators and parasites for at least the major pests, namely

*Empoasca on beans much better repudiated
than other pests*

the Mexican Bean Beetle, the Bean Pod Weevil and the Bean Leafhopper (1). Resistent varieties of bean to leafhoppers were developed in Mexico as early as 1957 (4) but it appears that these very valuable developments were never pursued. Presumably similar discontinuity has occurred in other countries of Latin America.

As a rule, there has been comparatively little published work on bean insects control in the last several years. Out of 194 articles on the subject, as reported by the Asociación Interamericana de Bibliotecarios y Documentalistas Agrícolas (AIBDA), from January to September 1974, only four (4) dealt with bean insects, three (3) with their biology, one (1) with their control. This is hardly believable when it is known that Phaseolus vulgaris lends itself so marvelously to scientific research.

If bean production has to keep its present status of domestic crop of comparatively small extension and

little financial return not allowing for protection by chemicals, it is imperative that biological and ecological studies be intensified so that yields can be maintained or increased. The basic biology of most of the pests has been worked out quite satisfactorily but their relationship with the varied environment, with the bean plant on which they are totally dependent, must be given increased attention. Only then will it be possible to work out satisfactory controls: biological, cultural or integrated with the chemical control.

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Table 1. Distribution of Phaseolus vulgaris L. in Central America

Country	Locality	Altitude (m)	Rainfall (mm)	Suitability for bean production	Species encountered	Life zone (Holdridge)
COSTA RICA	Turrialba	600	3,500	Not suitable	<u>E</u> <u>phaseola</u> ^a	Premontane moist forest
	Paraiso	1,450	2,000	Not suitable	<u>E</u> <u>phaseola</u> ^a	Premontane moist forest
	Cervantes	1,375	2,000	Not suitable	<u>E</u> <u>kraemeri</u> , <u>phaseola</u> , <u>arator</u> ^b	Subtropical very wet forest
	San Antonio	800	1,500	Very suitable	<u>E</u> <u>kraemeri</u> , <u>phaseola</u> , <u>arator</u> ^b	Subtropical rain forest
	Canas	100	1,000	Suitable	<u>E</u> <u>kraemeri</u> , <u>arator</u> ^b	Tropical dry forest, moist province transition
	Alajuela	1,200	2,000	Very suitable	<u>E</u> <u>kraemeri</u> ^a , <u>arator</u> ^b , <u>phaseola</u>	Subtropical humid forest
	Liberia	300	1,750	Very suitable	<u>E</u> <u>kraemeri</u> ^a , <u>arator</u>	Premontane moist forest, basal belt transition
	San Isidro del General	-	2,500	Very suitable	<u>E</u> <u>kraemeri</u> ^a	Tropical moist forest
	Cartago	2,300	2,500	Suitable	<u>E</u> <u>phaseola</u>	Premontane moist forest
NICARAGUA	Matagalpa	900	1,300	Very suitable	<u>E</u> <u>kraemeri</u> , <u>arator</u> , <u>phaseola</u> ^b	Tropical dry forest, moist province
	Somoto	750	1,250	Suitable	<u>E</u> <u>kraemeri</u> ^a	Tropical dry forest
	Esteli	900	1,000	Very suitable	<u>E</u> <u>kraemeri</u> , <u>arator</u>	Tropical dry forest

^a Numerically more abundant or exclusive

^b Abundant

Table 1. (continued)

Country	Locality	Altitude (m)	Rainfall (mm)	Suitability for bean production	Species encountered	Life zone (Holdridge)
EL SALVADOR	San Andres	500	1,800	Suitable	<u>E. kraemeria</u> ^a , <u>phaseolab</u>	Subtropical wet forest
	Apopa	600	2,000	Suitable	<u>E. kraemeria</u> , <u>phaseola</u>	Tropical dry forest
GUATEMALA	Las Moritas	500	2,100	Very suitable	<u>E. kraemeria</u> ^a , <u>phaseolab</u>	Tropical dry forest, moist province transition
	Jutiapa	900	2,000	Very suitable	<u>E. kraemeria</u> ^a , <u>phaseolab</u> , <u>arator</u>	Subtropical dry forest
	Chimal- tenango	1,950	1,500	Not suitable	<u>E. phaseolaa</u> ^a , <u>kraemeria</u>	Premontane wet forest
HONDURAS	Danli	800	1,400	Very suitable	<u>E. kraemeria</u> , <u>phaseola</u>	Subtropical wet forest
	Zaragoza	-	800	Suitable	<u>E. kraemeria</u> , <u>phaseola</u>	Subtropical dry forest



LAS PLAGAS DEL FRIJOL EN CENTROAMERICA Y SU COMBATE

Leonce Bonnefil

INTRODUCCION

De fines de noviembre a principios de diciembre de 1964 tuve la oportunidad de tomar parte en un recorrido por America Central con el propósito de observar las condiciones del cultivo del frijol. Mi tarea en términos generales fue el reconocimiento de las plagas la determinación hasta donde fuera posible de su distribución la evaluación de los daños causados por ellas y el inventario de los medios de combate. Las fuentes de información que utilizamos fueron conversaciones con técnicos de agencias gubernamentales y particulares, agencias de extensión fin que las observaciones personales en el campo y por último la revisión de material técnico de divulgación. Me complace dar las gracias en esta oportunidad a varios técnicos que con mucha gentileza me proporcionaron datos en proceso de recolección o análisis y que no habían sido aun publicados.

No se esperaba que este reconocimiento fuera exhaustivo puesto que tales trabajos por lo general exigen mucho más tiempo del que dispusimos. Sin embargo se lograron algunas observaciones preliminares que pueden servir de guía para un programa de investigación.

Estas observaciones fueron las siguientes:

- 1 hay bastante incertidumbre en cuanto a la identidad de los insectos que dañan el frijol en Centroamerica
- 2 se sabe relativamente poco de la biología y de la ecología de muchos de estos organismos
- 3 los medios de control no son uniformes y pueden derivarse grandes ventajas de la confrontación de los métodos probados en los diferentes países con el fin de obtener un método a la vez eficaz práctico y económico
- 4 además del control químico parecería que debe introducirse otros medios de control, a sea para reemplazar o para ayudar a este. Por ejemplo meo es prácticas culturales variedades resistentes control específico para la protección de organismos benéficos etc.

En esta charla se presentan las informaciones disponibles por pocas que sean en relación con las observaciones enumeradas anteriormente. Se dan a conocer los esfuerzos desplegados para mejorarlas, a sea por continuación de instituciones dedicadas a

trabajar el frijol o a través de ensayos que hemos iniciado en el Instituto Interamericano de Ciencias Agrícolas.

En primer lugar creo útil identificar brevemente los organismos que encontramos apuntando particularidades interesantes para su conocimiento y eficaz combate. Luego hablaremos de los métodos de control observados terminando con una discusión breve sobre los trabajos que se están llevando a cabo en el Centro de Enseñanza e Investigación del IICA en Turrialba.

PLAGAS IMPORTANTES DEL FRIJOL EN AMERICA CENTRAL

De los 90 y más insectos que atacan al frijol en la America Latina 78 se encuentran con cierta frecuencia en Centroamerica y no más de unos 15 de ellos son realmente de importancia económica.

De este numero algunos insectos son muy conocidos y como tambien ocurre en otras regiones son responsabilizados de daños quizas mayores de los que en realidad causan otras al contrario son poco conocidas o ignoradas aun cuando sus depredaciones son de gran importancia. Se cree que la causa de este error es que no siempre los daños espectaculares son los mas serios. Una comparación típica es la de las vaquitas y de los saltahojas.

Por otra parte los nombres vulgares se prestan a muchas confusiones. Sin entrar en muchos detalles de taxonomía el mismo nombre cubre tipos muy diferentes. Al contrario el mismo organismo recibe distintos nombres en los varios países. La clasificación taxonómica de los tipos centroamericanos es incompleta y los especialistas estan revisandola actualmente.

En la rapida descripción que haremos de las plagas tratare de reunir todo los nombres comunes y dar a conocer las identificaciones mas recientes.

La conchuela tortuguilla, vaquita o caratana (*Epilachna varivestris*) es un insecto coleoptero de la familia Coccinellidae.

Es una plaga bien conocida y ampliamente distribuida en Centroamerica. Sus danos son bastante típicos aunque algunas veces pueden confundirse con los de ciertos gusanos o de crisomelidos sobre todo cuando esos danos son recientes. Por lo general son muy extensos pero pueden variar mucho de region a region, en la misma region de un lugar a otro o de una época a otra. Según Eddy McAllister (1) las condiciones secas, calidas limitan el desarrollo de una población alta de este insecto o reducen los danos.

Se ha establecido que el aumento de humedad y temperatura durante el verano hacen que el insecto salga de los lugares donde paso su estado de larva. Tanto las larvas como los adultos se alimentan del follaje del frijol por la cara inferior. El tejido vegetal es devorado en bandas paralelas. Cuando el daño esta muy avanzado solo quedan partes de la epidermis superior y las nervaduras principales y las hojas toman entonces un aspecto esqueletizado.

Las larvas ocasionan mas danos que los adultos una larva de gran tamaño puede consumir hasta una hoja al día. Sabemos de casos de infestacion en los cuales no solo las hojas sino el tallo las flores y las vainas son devoradas.

La *Epilachna varivestris* es un gorgojo de forma hemisférica de color amarillo, de 1/4 a 1/3 de pulgada de largo y 1/5 de pulgada de ancho con ocho puntos negros de cada elito dispuestos en tres lineas a traves del cuerpo del insecto. Las larvas completamente desarrolladas muestran 6 hileras de espinas bifurcadas con puntas de color negro. Son de forma ovalada de color amarillo. Se alimentan activamente durante unas 2 o 5 semanas y luego se convierten en pupa en la manera característica de los coccinelidos generalmente en el envés de la hoja del frijol o de otra planta hospedera. La larva cementa su parte posterior a la superficie foliar no danada fuerza la piel pupal a replega sobre el abdomen y deja expuesta la parte anterior que aparece lisa redonda y de color amarillo. El insecto pasa hasta 10 dias en este estado pupal. El ciclo entero dura un mes como promedio.

El picudo del ejote (*apion godmani*) es un coleoptero de la familia Curculionidae. Este insecto puede bajo ciertas condiciones y en ciertas areas ser considerado como una plaga de suma importancia puesto que puede llegar a impedir el cultivo del frijol.

Se debe hacer notar (5) que muchas veces a la especie *A. godmani* se agrega la *A. aurichalceum* siendo ambas especies muy parecidas. *A. godmani* es de mayor importancia ocasionando daños mas extensos aunque no siempre se encuentra en mayor abundancia. Los huevecillos de *A. godmani* son puestos individualmente en casi todos los granos de las vainas y las larvas al desarrollarse consumen capullos separados mientras que en el caso de *A. aurichalceum* las larvas son gregarias se desarrollan sobre un numero menor de granos y pupan en un capullo de varios compartimientos.

El ataque del picudo empieza con la floracion y el ciclo biológico del insecto se desarrolla paralelamente al crecimiento de la vaina. Los adultos son muy pequeños, miden de 2.50 a 2.80 mm son de color negro con unos pelos blancos.

El adulto se alimenta del follaje flores y vainas y generalmente se puede ver en la parte inferior de las hojas o alrededor de las flores o las vainas y de los granos. Una sola larva puede destruir una semilla entera si es joven o puede dañar semillas maduras que pierden por este hecho calidad comercial. Las vainas a veces son flaccidas y presentan depre-

siones de color amarillo. Las vainas maduras cuyos granos han sido reemplazados por las cubiertas de capullos vacios aparecen marchitas y muestran los agujeros por los cuales salieron los adultos.

El desarrollo de la poblacion de picudos esta mas relacionado con el estado de desarrollo de las vainas que con las variaciones climaticas. La infestacion se manifiesta cada año en la misma epoca y las fluctuaciones del grado de infestacion de año a año probablemente son debidas a la influencia de factores ambientales como la siembra o cambios en las variedades usadas etc.

La denominacion vulgar de vaquitas conchitas o doradillas abarca varios insectos casi todos coleopteros de la familia Chrysomelidae, de los generos *Cerotoma*, *Andrector*, algunas veces *Diabrotica* y probablemente otros. Considerando primeramente las vaquitas *Cerotoma* se conocen las especies *C. atrofasciata*, *C. salvini* y *C. ruficornis* que son probablemente las de mayor difusion por lo menos en Costa Rica. La clasificacion completa y definitiva no ha sido hecha aun. En nuestro recorrido por Centro America encontramos un gran numero de tipos que estamos clasificando.

La especie de *Diabrotica* que aparentemente se encuentra con mayor frecuencia en la region es *D. balteata* sobre todo a alturas menores de 2 000 metros sobre el nivel del mar. El insecto tiene un caracteristico paton verde con cuatro bandas transversales amarillas. Mide aproximadamente 4.4 mm de longitud y 3.1 mm de ancho. Como la *Cerotoma* la *Diabrotica* pone sus huevos en el suelo y las larvas se alimentan de las raíces.

Sin embargo todos estos gorgojos tienen caracteres morfológicos similares los mismos hábitos y ocasionan danos semejantes. Por lo general (4) varían mucho en color y dibujos mide 1/5 de pulgada hasta 1/4. Muy a menudo toman una actitud de reposo en el envés de las hojas y se dejan caer al suelo si son perturbados. Tienen un porcentaje de reproduccion bastante alto. Los huevos dan nacimiento a larvas pequeñas oelgadas de color blanco que se alimentan de raíces del frijol. El estado de pupa lo pasa en el suelo en una celula de tierra a una profundidad de mas o menos 4 pulgadas.

El daño causado por la *Cerotoma* al follaje se presenta en forma de huecos mas o menos redondos. Es muy facil confundir este dano con el de la doradilla (*Diabrotica*) aunque esta ultima especie tiende a comer el borde de las hojas o el peciolo algunas veces separandolas de la mata del frijol.

Los adultos devoran muchas veces el tallo de las matas jóvenes exponiendolas al ataque de bacterias y hongos.

Las depredaciones de las vaquitas y doradillas influyen mucho en el rendimiento al reducir el área foliar y consiguientemente la superficie fotosintética y dañar el sistema radial. El dano es particularmente importante cuando la mata es joven. Los suelos sueltos y húmedos favorecen la oviposición y el desarrollo de las larvas. En periodos secos los huevos se desecan la canchales de tierra que rodea las pupas.

desintegra y estas quedan expuestas a toda clase de peligros.

El minador de la hoja (*Liriomyza* sk.) pertenece al orden Diptera y a la familia Agromyzidae. Sus larvas minan el mesodermis de las hojas. Estas larvas forman hilos en forma cilíndrica pupan en el suelo a poca profundidad. El adulto es una mosca de color negro con patas largas y delgadas, una cabeza más larga que ancha. Las hembras ocasionan un daño limitado alimentándose de las hojas.

El daño es más importante cuando las plantas son tiernas y sólo en circunstancias especiales alcanzan un nivel peligroso. Por lo general el daño es menor en portales cuando el suelo es fértil y tiene una amplia cantidad de materia orgánica. La humedad del suelo favorece un crecimiento rápido de las plantas y previene el ataque de los minadores.

La mosca blanca es un homoptero de la familia Aleyrodidae. Como lo indica el nombre su color blanquecino pero no es una mosca. El color blanco se debe a unas secreciones con textura de cera semejantes a hilos delgados y rizados producidas por formas juveniles que imparten un aspecto lanoso al individuo. Al contrario de otros homopteros exudación azucarada no cae del insecto sino que se reúne en globulos largos sobre su cubierta lanosa. Los huevos son puestos en círculos en el envés de las hojas especialmente las más viejas. Las ninfas se transforman en imagos dejando su piel pegada a la superficie foliar.

La mosca blanca se encuentra en casi todos los ambientes. No se sabe con certeza la magnitud del daño que pueden ocasionar poblaciones grandes de este insecto. Por lo menos no produce daños visibles. Se debe recordar sin embargo que estos insectos son vectores de enfermedades virales en ciertos cultivos como el algodón y que pueden actuar similarmente en el frijol.

El chinche verde (*Mezara viridula*) y otros homopteros de la misma familia Pentatomidae se encuentran en el frijol pero nunca en gran número al igual que pulgones del género *Aphis*. De manera general estos insectos chupadores no ameritan un tratamiento especial en plantaciones comerciales donde se llevan a cabo aplicaciones para el control de plagas de mayor importancia.

El gusano peludo (*Estigmene acrea*) es un lepidoptero de la familia Arctidae. Puede constituir un problema muy serio para los productores de frijol cuando frecuentes las infestaciones fuertes y es tan voraz que puede destruir completamente el follaje y afectar el rendimiento.

Los gusanos del género *Estigmene* constituyen una plaga. Las larvas miden en promedio 5 cm. son de color café o negro con líneas amarillas. El adulto es una mariposa que tiene las alas anteriores blancas con unos puntos negros y las alas traseras blancas. La hembra y anaranjadas en el macho.

La siembra del frijol es muy a menudo atacada por varios gusanos que salen del suelo en la noche y se esconden al amanecer. Todos son lepidopteros de la familia Noctuidae y pertenecen a varios géne-

ros: *Heliothis*, *Laphygma*, *Prodenia*, *Feltia*, *Agrotis*. El daño varía con las especies de un año al otro. Algunas especies se limitan a cortar las plantitas al nivel del suelo y comen muy poco de ellas otras suben al follaje y devoran casi toda la superficie foliar otras permanecen en el suelo y comen las raíces.

El ciclo biológico depende de la especie. Unas especies se desarrollan en pocas semanas otras requieren casi un año. Con pocas variaciones el método de control es el mismo para todas.

Las chicharritas conocidas también como saltahojas son una plaga de suma importancia en todas las regiones donde se cultiva el frijol. La Chicharrita verde es de interés particular un insecto homoptero de la familia Cicadellidae, del género *Empoasca*.

Por lo general los saltahojas chupan la savia de las hojas las cuales muestran una multitud de puntos blancos pequeños. Es muy posible que en esta forma los insectos puedan influir sobre el desarrollo normal de la planta y por consiguiente sobre su producción.

Síntomas similares son característicos también de otras especies de *Empoasca* tales como *E. maligna*, *E. abrupta*, *E. filamenta*, *E. bifurcata*, etc. Otras como *E. fabae*, *E. mali*, *E. phaseoli* no se alimentan del mesófilo sino del floema o sea del tejido conductor de savia y ocasionan otro tipo de daño. Las hojas tiernas se arrugan, se empiezan a amarillar por los bordes y luego se secan. El desarrollo general de las plantas se reduce y la producción de vainas es casi nula. No hay evidencia que sean inyectados sustancias tóxicas a la planta aunque ha sido demostrado que *E. Solani* inyecta dióxido de carbono y posiblemente invertebra en una dieta artificial. Parece muy posible que los daños al sistema de circulación de savia impiden el desarrollo normal de los tejidos en el envés de la hoja lo que ocasiona el arrugamiento.

No se ha verificado la transmisión de virus por saltahojas pero es posible que las plantas atacadas por esos insectos sean más susceptibles a algunas enfermedades criptogámicas.

La chicharrita pasa todo su ciclo biológico sobre la mata de frijol. La duración de este ciclo parece variar con los factores ambientales al igual que la expresión de los síntomas siendo estos más severos en lugares calientes. En lugares poco elevados los insectos son muy activos se reproducen más rápidamente y como consecuencia dañan más las matas de frijol.

En nuestras parcelas experimentales en Turrialba hemos observado un parasito del frijol muy poco común es un insecto lepidoptero que pertenece a la familia Ctenopseidae y al género *Lasneysia*. Según el Dr. D. Davis de la U. S. National Museum es posible que la especie sea nueva. El insecto ha sido observado hasta en el 30% de las matas. Estamos actualmente en proceso de determinar el ciclo biológico del parasito y por eso no tenemos datos muy exactos. Las larvas se encuentran generalmente en el cuello de las plantas a poca profundidad sobre la corteza. El cuello de las matas parasitadas se ensancha

considerablemente

La presencia de las larvas y pupas en los tejidos no afecta el crecimiento de la planta ni interfiere con la producción solo en ciertas ocasiones la planta se pone amarilla / muere y las vainas ya no maduran

Comunicación personal

PLAGAS OBSERVADAS EN LOS PAISES DE AMERICA CENTRAL

El recorrido que hicimos por los países centroamericanos tuvo lugar al final del periodo lluvioso y el tiempo ya estaba bastante seco. Durante las dos semanas que duro el viaje llovió solo un día cerca de la frontera de Nicaragua / Costa Rica. La siembra de postrema acababa de madurar en la mayoría de las zonas o ya se había cosechado. No era la época más indicada para observar las plagas que atacan temprano al frijol (gusanos cortadores, pulgones, minadores, etc.). De todos modos la opinión unánime es que la primera siembra es la que generalmente sufre mayores daños y la mejor época para estudiar las plagas sería en mayo o a más tardar abril. La información sobre la primera siembra fue obtenida a través de publicaciones o contactos con otros técnicos.

El primer país que visitamos fue Nicaragua. En las parcelas de frijol de la Estación Experimental de La Calera del Ministerio de Agricultura y Ganadería encontramos una infestación muy fuerte del gusano cogollero (Estigmenon), también de gusanos cortadores (Feltia) de gusano de algodón (Alabama) y mosca blanca (Aleyrodidae). En Jinotega (450 metros sobre el nivel del mar) coleccionamos chicharritas (Empoasca) también en Matagalpa y Jinotega (1 020 metros). En estas regiones son bastante lluviosas y muy poco pobladas. Los cultivos predominantes son la papa, las naranjas y el café. Las regiones frijoleras de Nicaragua son de Esteli, Matagalpa hacia la frontera con Honduras. Son regiones de elevaciones medianas (1 020 metros) o bajas (560 metros) con 800 a 1 000 mm de precipitación. La siembra de primera que se cosecha en julio según testimonio de la gente sufre menos de las plagas. El frijol se cultiva en escala comercial y una buena parte de la cosecha se exporta. El endimio es bastante alto y se hace uso de insecticidas (en Esteli los productores espolvorean con Metilico).

En la región de Santa Ana en El Salvador encontramos una infestación muy alta (40% o más) de picudo (Apion). Las plantaciones estaban en estado muy avanzado de madurez pero sin embargo se podía ver gran número de chicharritas. La manifesta-

ción del daño por estos insectos varía mucho sobre todo con el tipo de frijol. El frijol de mata en la región de Santa Ana muestra bastante arrugamiento y poco amarillamiento. El frijol de guía solo reveló un puntado blanco sin ninguna deformación de las hojas.

La mosca blanca (Aleyrodidae) era muy abundante. El Centro Nacional de Agronomía en Santa Tecla está efectuando ensayos para el control de este insecto también está trabajando sobre la posible transmisión de virus por las chicharritas (Empoasca).

La primera región visitada en Guatemala fue Parramos a 1 820 metros de altura, seca y fría. Las explotaciones son bastante grandes y se obtienen dos cosechas al año. La primera es la más dañada por las plagas.

Se encontró doradilla, conchuelas, chicharritas, la mosca blanca y pulgones. Los síntomas del daño se manifestaban muy levemente. En Chimaltenango los daños fueron mucho más visibles especialmente los ocasionados por chicharritas y vaquitas. En la Estación Experimental del IAN (Instituto Agropecuario Nacional) habían algunos ensayos para el control de vaquitas y de la doradilla.

Se visitó después la región de Jutiapa, seca y desolada. Se observaron pocos frijoleros y se efectuaron dos colecciones a alturas de 760 y 480 metros. El picudo (Apion) y la chicharrita (Empoasca) fueron las especies predominantes. A pesar del calor el daño causado por Empoasca no fue típico.

En la Escuela de Agricultura de Barcenás eran muy abundantes las vaquitas, chicharritas y moscas blancas. Se notó un achaparramiento muy intenso y un amarillamiento de las hojas tiernas. Alrededor del Lago Amatitlán se siembra algo de frijol tanto de mata como de guía. Las siembras parecían bastante sanas a pesar de que habían presentes chicharritas. No se observó evidencia de daño.

En Honduras con la valiosa ayuda de los técnicos de la Escuela Agrícola Panamericana fue posible visitar las zonas de Danlí (820 metros) y El Paraíso (780 metros). Son regiones aparentemente muy apropiadas para el cultivo del frijol. En realidad observamos muchas siembras de buen tamaño pero pocos insectos, únicamente algunas vaquitas y chicharritas. Los daños eran poco visibles.

Provisionalmente se presenta el cuadro N° 1 sobre la incidencia de 12 plagas del frijol en los cinco países centroamericanos. Se puede constatar que los saltaños son la plaga más frecuente seguidos en importancia por las vaquitas y la mosca blanca.

CUADRO Nº 1

INCIDENCIA RELATIVA DE LAS 12 PRINCIPALES PLAGAS DEL FRIJOL EN AMERICA CENTRAL SEGUN OBSERVACIONES PERSONALES Y DATOS PUBLICADOS

	Gusanos cortadores (Feltia, Prodenia, etc)	Vaquitas doradillas (Cerooma Andrictor Diabrotica)	Minador (Chalepus)	Tortuguito (Epilachna)	Picudo del ejote (Apion)	Chicharritas (Empoasca)	Pulgones (Aphis)	Mosca blanca (Aleyrodidae)	Minador (Liriomyza)	Gusano peludo (Eugimene acraea)	Gusanos del follaje (Alabama etc)	Barrerador del cuello (Laspeyresia)
COSTA RICA	3	4	1	1	1	4	1	2	1	1	2	2
EL SALVADOR	—	3	—	1	3	4	1	2	4	—	2	—
GUATEMALA	1	2	1	4	3	4	2	2	1	2	—	1
HONDURAS	1	3	1	1	4	4	1	3	—	—	—	—
NICARAGUA	2	3	1	3	1	3	1	3	2	3	2	—

— insectos ausentes
1 muy pocos
2 pocos

3 insectos numerosos
4 muy numerosos

ESTADO ACTUAL DE LA LUCHA CONTRA LAS PLAGAS DEL FRIJOL EN CENTROAMERICA

Por regla general el productor de frijol en América Central no combate los insectos que invaden su cultivo. Es obvio que nota y aprecia las pérdidas que ocasionan las plagas, su actitud negativa ante ellas se puede explicar por varias razones:

El cultivo de frijol con excepción de ciertas partes de Guatemala, Honduras y Nicaragua es una actividad de carácter familiar. El tamaño de las explotaciones es muy pequeño, su promedio es de 1 a 14 a 20 manzanas*.

El producto de la siembra es primordialmente para consumo doméstico y se venden solamente los sobrantes. Algunas veces se conserva una cantidad de semilla para siembras futuras.

En los países en que se cultiva el frijol para la exportación, el productor no exporta sino que vende sus cosechas a un intermediario. Su explotación, aunque tiene entonces un carácter comercial, es de tamaño reducido de modo que no justifica la compra de equipo e insecticidas para el combate de las plagas. El intermediario no parece preocuparse por el rendimiento puesto que su negocio se limita a comprar el producto de varios agricultores y a revenderlo con ganancia.

Por otra parte, el frijol tiene un periodo vegetativo muy corto y el uso de pesticidas debe hacerse

* Una manzana 6988 96 metro cuadrados

a tiempo y en concentraciones exactas. Por consiguiente el combate para ser eficaz y económico requiere bastante conocimiento y disciplina de parte del agricultor.

La situación se complica porque, debido a los riesgos característicos del cultivo el precio de los granos está sujeto a mucha fluctuación. El productor al no poder influir sobre el precio de su producto en el mercado siempre es reticente e inerte lo menos posible en su explotación.

Solo el productor en gran escala tiene interés en aumentar los rendimientos por medio del combate de las plagas. En las regiones donde las explotaciones son bastante extensas y de carácter comercial los insectos y enfermedades se combaten con cierto grado de éxito y por esto los rendimientos superan los de las explotaciones familiares de manera muy significativa.

Los productos y las concentraciones utilizadas en el combate de las plagas varían mucho de un lugar a otro. Los servicios de extensión y las agencias de firmas productoras de pesticidas publican folletos de divulgación con recomendaciones. Más valiosas son las pruebas que llevan a cabo los organismos oficiales de los diferentes países centroamericanos en sus estaciones experimentales.

En Nicaragua tuvimos la oportunidad de visitar un ensayo para el control del gusano peludo en El Salvador, uno para el control de la mosca blanca y en Guatemala otro contra vaquitas y chicharritas. Los resultados de estos ensayos serán publicados probablemente en el curso de 1965.

TRABAJOS RECIENTES SOBRE CONTROL DE PLAGAS DE FRIJOL

En la revisión de los trabajos sobre control de plagas efectuados en el Continente Americano y África se puede observar que del año 1946 al presente, se ha publicado comparativamente poco acerca del combate de los insectos del frijol con excepción de la conchuela (*Epilachna*).

Los primeros trabajos que se llevaron a cabo en los Estados Unidos de América y México, establecieron que este insecto era muy susceptible a las aplicaciones de Roerona. En mezcla con piedra pomez y azufre este producto dió un control absoluto (100% de mortalidad). En aquella oportunidad el Parathion, Toxafeno Dieldrin Sevin eran considerados como prometedores. En 1951 la conchuela mostró resistencia a la Roerona y se reemplazó entonces por el Malathion que era muy eficaz y tenía una toxicidad bastante baja para los mamíferos. Por muchos años el Malathion fue el tratamiento clásico hasta que a su vez, fue desplazado por otros productos sumamente eficaces tales como el Clorfen el Dieldrin el Aldrin el Dieldrin el Thiodan y el Sevin. Este último insecticida por su grado de eficacia muy consistente, su toxicidad baja para los mamíferos y su prolongado

efecto parece ser el producto por excelencia para el control de la *Epilachna*.

Mc Kelvev y Cuevara y Cortes en 1946 recomendaron en México el DDT y el BHC como los mejores productos en el combate del picudo (*Apion godmani*) aplicados en forma de polvo humedecible en el periodo de floración. Estos mismos técnicos efectuaron ensayos subsiguientes utilizando Parathion DDT Metoxicloro Clordano EPN Aldrin Dieldrin y Dilan. El DDT y el Parathion resultaron ser los productos más eficaces.

J. Guevara Calderón (6) propuso una combinación de variedades resistentes y la aplicación de insecticidas como el método de control más eficaz. La variedad Pinto 168 que es altamente resistente (muestra solamente 8.15% de infestación), no requirió aplicación de insecticidas, en cambio Mex 228-7 (15% a 45% de infestación) necesitó una aplicación, Pue 152 moderadamente susceptible (45% a 100% de infestación) Dgo 224-42 y Ch 551-11 que son susceptibles necesitaron dos aplicaciones de Folidol para producir económicamente y la variedad Negro Mecentral altamente susceptible necesitó más de dos aplicaciones.

La chicharra *Emmiasca* fue primeramente controlada con caldo bordelés, azufre o piretro. Luego se comprobó que la Sabadilla era más efectiva. Grovovsky en 1914 descubrió la gran efectividad del DDT. Posteriormente al Toxafeno el Demeton el Metoxicloro el Parathion resultaron muy eficaces. En trabajos más recientes Metasystox y Labacyd han dado excelente control. El sistémico Thimet (Phorate) ha dado control absoluto con una sola aplicación al sembrar el frijol en Guanacaste Costa Rica. Iguales resultados se obtienen con Di-Syston. En el Brasil H. Vaz de Arruda (1) ha demostrado que la aplicación combinada de DDT y Metasystox supera la acción aislada del sistémico posiblemente debido a la acción acaricida de Metasystox. El DDT supuso al Endrin y al Diazinon. Es interesante notar que A. Suplicy y M. Fadigas (8) obtuvieron resultados significativamente mejores con Di-Syston que con Thimet.

El minador *Liriomyza* era combatido anteriormente con sulfato de nicotina aceite y agua. Luego se verificó que el DDT el Toxafeno, el Diazinon, el Dieldrin y el Parathion controlaron el insecto satisfactoriamente. *Liriomyza* puede desarrollar resistencia al DDT.

El tratamiento de las semillas con Endrin Dieldrin Aldrin proporciona control completo sin efectos dañinos a la germinación. No se ha establecido cual es el mecanismo de este efecto.

Muy escasa es la investigación sobre el control de las plagas menos importantes del frijol. En el Cuadro Nº 2 se han condensado las recomendaciones para el control químico de las principales plagas del cultivo. Esos datos fueron tomados en parte de las Recomendaciones sobre insecticidas publicadas por la División de Investigaciones Entomológicas del Departamento de Agricultura de los Estados Unidos y en parte del informe final de la Primera Asamblea

CUADRO N. 2

TRATAMIENTOS PARA LAS PRINCIPALES PLAGAS DEL FRIJOL

Plaga	Insecticidas	Libras de sustancia activa por acre	Forma de aplicación	Precauciones
Conchuela (-puachna)	Rotenona	0.25	P E	Solo por personas con experiencia
	Malathion	1.25	P PH	
	Parathion	1.5	P PH	
	Toxafeno	2	P PH	
	Sevin	1	P PH	
	Metoxyclo	1.5	P E	
	Dieldrin	0.5	P PH	
	Diazinon	0.75	PM	Las plantas tratadas son peligrosas para el ganado
	DDT	0.75	C P PH	
	Thimet	1	G	Thimet y Disyston debajo de las semillas (no en contacto con ellas)
	Disyston	1	G	
vaquitas doradilla (Ceratomyza, Andrector Diabrotica)	DDT	1	P PH E	Las plantas tratadas son peligrosas para el ganado
	Sevin	1	P PH	
	Rotenona	0.25	P E	
	Malathion	1.25	P PH G	
Arañadores (Liriomyza, Chalepus)	Malathion	1.25	PH E	
	Diazinon	0.75	PH E	
Pulgones (Aphis)	Malathion	1	P PH E	Solo por personas con experiencia
	Disyston	0.5	E	
	Diazinon			
Saltahojas (Empoasca)	Parathion	1	P PH E	Las plantas tratadas son peligrosas para el ganado
	DDT	0.75	P PH E	
	Sevin	1	P PH E	
	Malathion	1.75	P PH E	
	Metoxyclo	1.5	P PH	
	Thimet	1.5	G	
	Disyston	1.5	G	
Gusanos cortadores (Feltia, Prodenia etc)	DDT	2	P PH E	Aplicar a la superficie del suelo antes de sembrar
	Dieldrin	2	P PH E	
	Toxafeno	2	P PH E	
Pudro (Apion)	DDT	1.5	P PH	Las plantas tratadas son peligrosas para el ganado
	Metoxyclo	1.5	P PH	
	Sevin	2	P PH	
Chirches (Nezara, etc)	Sevin	2	P PH	Las plantas tratadas son peligrosas para el ganado
	DDT	1	P PH E	

P=polvo seco
G=granulos

PM=Polvo humeante

E=concentrado emulsificable

latinoamericana de Fitoparasitología (México) y co- tiene además algunos datos personales.

OTROS MEDIOS DE COMBATE

Con pocas excepciones los frijoles que visita mos no estaban bien cuidados. Pocas explotaciones usaron fertilizante. La semilla era de dudosa calidad y no recibió ningún tratamiento en la mayoría de los casos. El desarrollo de las malas hierbas casi inmediata el crecimiento de las plantas de frijol. Es indudable que estas condiciones son ideales para el desarrollo de las plagas y enfermedades que contribuyen a disminuir aun más el rendimiento. Unas buenas prácticas culturales pueden contribuir decididamente a la protección del cultivo.

Además de la lucha por medios mecánicos y químicos algunos de las plagas del frijol sufren la acción de ciertos organismos parásitos. Una mosca de la familia Tachinidae, *Phococera claripennis* pone sus noviecillos blancos sobre la larva de la conchuela *Epilachna*. Por lo general solo una larva de la mosca completa su desarrollo. El grado de parasitismo es por eso bastante bajo. Hay otra mosca Tachinidae (*Paradores epilachinae*) que puede ser utilizada en un programa de control biológico.

Además las larvas y pupas de la conchuela sirven de alimento a varios insectos predadores. Muy pocas enfermedades bacterianas infectan este insecto y no se conocen parásitos internos.

La larva del *Apion* es parasitada por una avispa pequeña del género *Triaspis*. No hay información exacta acerca de la eficacia de este parásito.

La posibilidad de utilizar variedades resistentes al picudo *Apion* y la chicharra *Empoasca* ha sido investigado en México por el Dr. José Guevara Calderón (7) y este método de combate es probablemente uno de los más deseables en Centroamérica.

Ultimamente (8) se ha probado el efecto de radiaciones Gamma y de la fertilización química sobre la conchuela *Epilachna*. Mucho y la larva del insecto fueron esterilizados con buen éxito. Hembras normales apareadas con machos esterilizados produjeron huevos estériles. Hembras esterilizadas apareadas con machos normales no produjeron huevos. Hembras normales apareadas con machos esterilizados produjeron huevos estériles pero apareadas luego con machos normales produjeron huevos normales.

CONCLUSIONES Y RECOMENDACIONES

De lo que precede se puede concluir que:

- 1) El cultivo del frijol en América Central es un cultivo domo en una pequeña escala.
- 2) Las condiciones de producción en general son desfavorables a un rendimiento máximo. Siendo las plantas débiles, expuestas a infección dañina de las plagas y enfermedades.
- 3) Sin embargo el frijol es la base de la alimentación del pueblo centroamericano y por eso la producción debe ser aumentada y mejorada.
- 4) Las plagas que atacan el frijol son bastante co-

nocidas en su biología pero no sucede lo mismo con su ecología, principalmente sus relaciones con la planta y por consiguiente la importancia de sus daños.

- 5) El combate de estas plagas ha sido estudiado en numerosos países con bastante éxito. Los adelantos técnicos logrados en las últimas décadas han puesto a disposición de los agricultores productos de eficacia más amplia. Es claro sin embargo que para ayudar eficazmente al agricultor latinoamericano se requieren métodos de lucha completos sencillos efectivos y baratos.
- 6) Dichos métodos deberían consistir en prácticas culturales o en la aplicación de productos químicos de resultados inmediatos que fueran reemplazados posteriormente por métodos biológico o por lo menos una combinación de métodos biológicos y químicos.

Con base a estas conclusiones me parece oportuno hacer algunas sugerencias a pesar de la poca experiencia que tengo en la región.

1) Por medio de intercambio de informaciones y de estrecha cooperación entre los varios organismos dedicados a la investigación debería hacerse un estudio cuidadoso de las plagas que incluya clasificación taxonómica, distribución geográfica, fluctuaciones anuales de las poblaciones, plantas hospedadoras, etc. Se sugiere que se estudie un plan progresivo y se confíe el mismo a una institución que se encargue de velar por la continuidad de los trabajos de investigación de la organización de la organización de los datos y finalmente de la publicación de los resultados.

2) Pueden organizarse dos tipos de trabajo. Uno como plazo que proporcionaría datos de necesidad inmediata por ejemplo sobre la economía o el control químico de las plagas. Otro a largo plazo que incluiría trabajo de educación más extensa por ejemplo en el cultivo y aprovechamiento de parásitos y predadores de desarrollo de variedades resistentes, etc.

En el IICA se han iniciado trabajos de ambos tipos. Por un lado se está estudiando el control químico de las plagas y se están llevando a cabo estudios biológicos. Por otro lado se están desarrollando variedades resistentes sobre todo a la chicharra. En particular respecto a este último proyecto estamos en el proceso de determinar las especies *Empoasca* asociadas con las plantas hospedadoras y el tipo del daño. Por otro tiempo estamos buscando en la colección de frijol del Instituto evidencias de resistencia con la esperanza de encontrar fuentes de resistencia que servirán posteriormente para el desarrollo de variedades resistentes.

El frijol es un cultivo que se presta con facilidad a muchos tipos de trabajo con un mínimo de tiempo y costo. Los que trabajamos en frijol tenemos la oportunidad inapreciable no solo de realizar trabajos científicos científicos y de alta calidad sino también de contribuir al mejoramiento de la cantidad y la calidad de un producto alimenticio básico.

OBSERVATIONS AND EXPLORATORY TRIALS

Collection of Empoasca

The first phase of my research on Central American Cicadellidae involved basic information on their taxonomic position, distribution and bionomics. During the three years of research, collections were made repeatedly at a great diversity of sites. The specimens were identified with the assistance of systematists of outstanding experience in that group of insects. Their determinations indicated that several species of Empoasca occur in the bean fields and on some common wild hosts, possibly with a predominance of E. kraemeri and E. phaseola. These species seem to distribute themselves in rather close correlation with the moisture gradient, kraemeri being mostly found in the dry locations and phaseola in the humid. Other Empoasca species of some significance were found to be prona, labialis, abrupta, and erigeron. Many specimens could not be named and were simply identified as belonging to large species complexes. Figure 6 shows where the different species were collected. Table 1 relates elevation and rainfall at stations nearest to the actual collection sites, specifies the suitability of these sites for bean culture, and indicates the life zone of Holdridge's ecological map. This last information is intended as a gross approximation to actual moisture indicators which require abundant and varied meteorological records, which, unfortunately, are not always available on account of the great diversity of the bioclimate of the region and the limited number of weather stations.

Where there is an alternation of dry and wet seasons, the two

Table 1 Distribution of Epigea on common bean (Phaseolus vulgaris L.) in Central America

Country	Locality	Altitude (m)	Rainfall (mm)	Suitability for bean production	Species encountered	Life zone (Holdridge)
COSTA RICA	Turrialba	600	3,500	Not suitable	<u>E. phaseola</u> ^a	Premontane moist forest
	Paraiso	1,450	2,000	Not suitable	<u>E. phaseola</u> ^a	Premontane moist forest
	Cervantes	1,375	2,000	Not suitable	<u>E. kraemeri</u> , <u>phaseola</u> , <u>arator</u> ^b	Subtropical very wet forest
	San Antonio	800	1,500	Very suitable	<u>E. kraemeri</u> , <u>phaseola</u> , <u>arator</u> ^b	Subtropical rain forest
	Canas	100	1,000	Suitable	<u>E. kraemeri</u> , <u>arator</u> ^b	Tropical dry forest, moist province transition
	Alajuela	1,200	2,000	Very suitable	<u>E. kraemeri</u> ^a , <u>arator</u> ^b , <u>phaseola</u>	Subtropical humid forest
	Liberia	300	1,750	Very suitable	<u>E. kraemeri</u> ^a , <u>arator</u>	Premontane moist forest, basal belt transition
	San Isidro del General	-	2,500	Very suitable	<u>E. kraemeri</u> ^a	Tropical moist forest
	Cartago	2,300	2,500	Suitable	<u>E. phaseola</u> ^a	Premontane moist forest
NICARAGUA	Matagalpa	900	1,300	Very suitable	<u>E. kraemeri</u> , <u>arator</u> , <u>phaseola</u> ^b	Tropical dry forest, moist province
	Somoto	750	1,250	Suitable	<u>E. kraemeri</u> ^a	Tropical dry forest
	Esteli	900	1,000	Very suitable	<u>E. kraemeri</u> , <u>arator</u>	Tropical dry forest

^aNumerically more abundant or exclusive

^bAbundant

Table 1 (continued)

Country	Locality	Altitude (m)	Rainfall (mm)	Suitability for bean production	Species encountered	Life zone (Holdridge)
EL SALVADOR	San Andres	500	1,800	Suitable	<u>E kraemeria</u> ^a , <u>phaseolab</u>	Subtropical wet for- est
	Apopa	600	2,000	Suitable	<u>E kraemeria</u> , <u>pha-</u> <u>seola</u>	Tropical dry forest
GUATEMALA	Las Moritas	500	2,100	Very suitable	<u>F kraemeria</u> ² , <u>phaseolab</u>	Tropical dry for- est, moist province transition
	Jutiapa	900	2,000	Very suitable	<u>E kraemeria</u> ^a , <u>phaseolab</u> ^b , <u>arator</u>	Subtropical dry forest
	Chimul- tenango	1,950	1,500	Not suitable	<u>E phaseola</u> ^a , <u>kraemeria</u>	Premontane wet for- est
HONDURAS	Danli	800	1,400	Very suitable	<u>E kraemeria</u> , <u>phaseola</u>	Subtropical wet for- est
	Zamorano	-	800	Suitable	<u>E kraemeria</u> , <u>phaseola</u>	Subtropical dry for- est

metrically more abundant species Kraemeria and phaeocola, constituted the populations and contained small numbers of the less predominant species. In the highlands going up toward the volcanic peaks, phaeocola gradually replaced Kraemeria up to a point where the host plants of the upper populations are no more encountered. In Guatemala, phaeocola was found rather abundantly in some cultivated bean patches, as high as 2000 meters.

Oviposition on Agricultural Plant Species

A cursory survey was made of the most common plant species growing spontaneously on or near farmsteads to determine whether they were used as hosts by empoidae leafhoppers. Aenistus rubroscens Schlecht., locally called "huaitito", Cestrum variegatum Klotzsch, an ornamental shrub, Rollinia and Dahlia, species of which are either grown as ornamentals or found in the wild state, volunteer potato plants (Solanum tuberosum L.), were examined for damage to the foliage or presence of

Both Aenistus and Cestrum are heavily infested (Figs. 7 and 8). These species are used as live post or hedges and obviously represent reservoirs of outstanding importance. Rollinia and Dahlia are not as widespread and probably are of lesser significance in the maintenance of populations of the leafhoppers. It was surprising that potato, although one of the most common bean plants, themselves severely attacked, showed no signs of damage and no eggs were located in the leaves and sections of stems. In search for wild hosts, I did not pursue any further although in the great diversity of tropical plants, a great many more species must serve as hosts to Empoidae leafhoppers.

Life History Study of Captive P. phiscolia

The second phase of the program was conducted at the Instituto Tecnológico de Costa Rica. This station is located on the Pacific coast, at an elevation of 600 meters. The basic plan was to develop several populations which could be manipulated according to needs. The first colonies to be established were of P. phiscolia from individuals collected on a wild solanaceous plant (Solanum elaeagnifolium) at Cartago, Costa Rica, on the Central Plateau, at an altitude of 2,300 meters (Fig. 8). From previous determinations, chances were that the population would be pure and not a mixture of species. Periodic samplings at that site showed that the conditions were undoubtedly favorable as evidenced by an ever-present dense population of P. phiscolia in a natural population as well as the captive one at the Research Center. These were frequently checked for purity. Reference specimens, dry on silica, were deposited with the Iowa Insect Collection.

Soon it was established that oviposition would occur in captivity on common bean. Other related plant species such as lima bean (Phaseolus lunatus L.), cowpea (Vigna sinensis Indl.), runner bean (Phaseolus coccineus L.), and peanut (Arachis hypogaea L.) were then tried.

Selected plant hosts for life history determinations had to be under protection from violent weather conditions and away from insects other than the species under study. A large greenhouse 30 m long, 10 m wide, and 15 m at the highest point of the slanted roof was built (Fig. 9). All four sides were covered with sarin green and the roof was of polyethylene film 0.3 mm thick. The structure was partitioned crossways into two equal sections, one to be used for chry-

production of plant material free from all insect contamination and the other to rear test insects. It was oriented in a general east-west direction for protection against the often violent rains and winds originating from the northeast. The floor was of coarse ground volcanic rock for rapid drainage.

The plants were grown in long wooden boxes, 9 m long, 60 cm wide, 45 cm high, the bottoms of which were perforated and garnished with a 5-mm layer of pebbles. All soil used in growing plants was fertilized with a nitrogen-rich formula of the type 20-10-10.

The wooden boxes were set on benches to ease observation and handling (Fig. 11). The insects were reared in cages 60 cm long, 60 cm wide, and 50 cm high (Fig. 13). The two lateral sides and the back were covered with screen screening. The upper half of the front was lined with plexiglass and the lower half was occupied by a sleeve of light cotton cloth. To feed the colonies of leafhoppers, selected bean species were grown in wooden flats 50 cm x 40 cm x 15 cm which could be introduced within the cages through the sleeve.

Records were kept of the temperature and moisture within the screenhouse with the help of a recording hygrothermograph. Typical readings for a complete year are listed in Table 2. Because the screenhouse was fairly tall, the hot air accumulated at a height which could not adversely affect either plant or insects. Even during the full raining the daily breeze shift from the northeast to the southwest, no harmful effect was ever recorded. In fact, up to a height of about 5 meters,

Table 2. Screenhouse humidity and temperature recordings at Turrialba, Costa Rica, for the period July 1966-June 1967

Week		Humidity		Temperature	
		Maximum	Minimum	Maximum	Minimum
July	4-11, 1966	93%	48%	30°C	18°C
	11-18, 1966	93%	48%	32°C	19°C
	18-25, 1966	94%	49%	31°C	18°C
	25-Aug 1, 1966	94%	52%	31°C	18°C
AVERAGES FOR JULY 1966		93.5%	49.3%	31°C	18.3°C
Aug.	1-8, 1966	95%	47%	31°C	17°C
	8-15, 1966	95%	44%	31°C	19°C
	15-22, 1966	94%	44%	33°C	19°C
	22-29, 1966	95%	47%	31°C	17°C
	29-Sept 5, 1966	94%	48%	31°C	19°C
AVERAGES FOR AUGUST 1966		94.4%	46.0%	31.4°C	18.2°C
Sept	5-12, 1966	95%	43%	32°C	18°C
	12-19, 1966	95%	43%	33°C	17°C
	19-26, 1966	94%	42%	32°C	18°C
	26-Oct 3, 1966	95%	40%	31°C	18°C
AVERAGES FOR SEPT 1966		94.7%	42.0%	32.0°C	17.8°C
Oct.	3-10, 1966	95%	38%	38°C	18°C
	10-17, 1966	95%	43%	38°C	19°C
	17-24, 1966	96%	40%	39°C	18°C
	24-31, 1966	94%	36%	38°C	19°C
	31-Nov 7, 1966	95%	50%	30°C	18°C
AVERAGES FOR OCT 1966		95.0%	41.4%	36.6°C	18.4°C
Nov.	7-14, 1966	95%	44%	30°C	15°C
	14-21, 1966	95%	44%	31°C	15°C
	21-28, 1966	95%	50%	30°C	17°C
	28-Dec 5, 1966	96%	41%	30°C	16°C
AVERAGES FOR NOV 1966		95.3%	44.8%	30.3°C	15.8°C

Table 2 (continued)

Week		Humidity		Temperature	
		Maximum	Minimum	Maximum	Minimum
Dec.	5-12, 1966	95%	53%	30°C	17°C
	12-19, 1966	91%	45%	29°C	17°C
	19-26, 1966	97%	44%	30°C	16°C
	26-Jan 2, 1967	95%	44%	38°C	18°C
AVERAGES FOR DEC 1966		93.8%	46.5%	31.8°C	15.8°C
Jan.	2-9, 1967	96%	34%	34°C	15°C
	9-16, 1967	97%	45%	34°C	17°C
	16-23, 1967	94%	40%	35°C	16°C
	23-30, 1967	95%	44%	38°C	16°C
	30-Feb 6, 1967	97%	40%	38°C	14°C
AVERAGES FOR JAN 1967		95.8%	40.6%	35.8°C	15.6°C
Feb.	6-13, 1967	96%	35%	37°C	15°C
	13-16, 1967	95%	40%	36°C	16°C
	22-27, 1967	96%	38%	35°C	15°C
AVERAGES FOR FEB 1967		95.7%	37.7%	36.0°C	15.3°C
Mar	1-6, 1967	94%	28%	36°C	14°C
	6-13, 1967	96%	33%	35°C	16°C
	14-20, 1967	97%	30%	35°C	14°C
	20-27, 1967	98%	30%	37°C	14°C
	27-Apr 3, 1967	97%	32%	36°C	15°C
AVERAGES FOR MAR 1967		96.4%	30.6%	35.8°C	14.6°C
Apr	3-10, 1967	95%	38%	32°C	18°C
	10-17, 1967	97%	43%	36°C	18°C
	17-24, 1967	96%	42%	37°C	18°C
AVERAGES FOR APR 1967		96.0%	41.0%	35.0°C	18.0°C

Table 2 (continued)

Week		Humidity		Temperature	
		Maximum	Minimum	Maximum	Minimum
May	1-7, 1967	97%	36/	37°C	16°C
	8-15, 1967	96%	42/	37°C	19°C
	15-22, 1967	97%	39%	37°C	17°C
	22-29, 1967	97/	42/	37°C	19°C
	29-June 5, 1967	97/	41/	36°C	18°C
AVERAGES FOR MAY 1967		96 1%	39 8%	36 8°C	17 8°C
June	5-12, 1967	96%	37/	36°C	18°C
	12-19, 1967	97%	40/	38°C	19°C
	-	-	-	-	-
28-July 4, 1967		95%	44/	38°C	18°C
AVERAGES FOR JUNE 1967		96 3%	41 3%	37 3°C	18 3°C

the temperature inside and outside the screenhouse differed by only 1 or 2 degrees. The roof and the sides did modify normal radiation, however, with the result that the plants were abnormally elongated, although they did not appear chlorotic or adversely affected in any other way

Building and plant boxes were kept regularly sprayed with Tedion to prevent establishment of phytophagous mites. The colonies of leafhoppers were often invaded by hordes of a small black-headed ant, Tapinoma melanocephalum Fab, which readily devoured the small nymphs. The benches supporting the cages had to be kept sprayed with a persistent insecticide, most commonly Aldrin. At one time, anthracnosis (Colletotrichum lindemuthianum Sacc and Mign) became a serious problem on our common bean plantings (Phaseolus vulgaris L) but was successfully eradicated thereafter by fumigation of the soil with methyl bromide prior to filling the troughs. In addition, to prevent the buildup of any pathogen, the soil at the end of each growing cycle was replaced with a freshly fumigated lot and the containers scraped and washed. Minor outbursts of angular leaf spot and powdery mildew could be controlled before they got out of hand.

Under field conditions it could easily be observed that certain cultivars of common bean were especially suitable host plants. In the interest of including a range of commercially grown legumes in the life history studies, legumes that are fast growing, of small growth habit, and readily accepted by leafhoppers were sought. The following

were selected

Kidney bean Phaseolus vulgaris L var S-19-N

Lima bean Phaseolus lunatus var Henderson baby lima

Cowpea Vigna sinensis

From a variety trial of some 300 entries in Honduras the kidney bean cultivar, S-19-N, well met the criteria above. In addition, it grew erect, was tolerant of diseases, and, except in mild attacks of powdery mildew, grew well and produced great amounts of foliage.

A sample lot of Henderson baby lima bean was obtained from the United States Department of Agriculture at Beltsville, Maryland. The variety proved suitable and large quantities of seeds could be purchased from commercial houses. Seeds of cowpea of a suitable, but unfortunately unidentified variety were available at the Turrialba Center in ample amounts.

Selection of Oviposition Site

Since the eggs of Phaseola are inserted into the plant where they are scarcely visible, it was necessary to discover which plant regions were used in oviposition. With this information it would be possible to develop appropriate techniques for collecting and observing the development of the egg stage.

The cowpea and lima bean were used in this test. Suspecting that the oviposition might vary with the stage of development of the plant host, potted seedlings, respectively 12, 15 and 24 days old, were used and 3 replicate plants were provided in each age group. Essentially, at 12 days the young plant has only its simple leaves but there are

fully developed. The 15-day plant has its first trifoliate leaf and a terminal shoot with rudimentary compound leaves. At 24 days of age, the plant has developed 4 trifoliate leaves, the terminal shoot is now a stringy leader with possibly one additional leaf bud, the simple leaves are still present and have not dropped.

The plants were caged from the time the seeds were sown. The cages were simple cylinders of inert plastic sheeting of such a diameter that they could be inserted into the soil of earthen pots 30 cm in diameter. The upper end of the cage was closed with fine nylon gauze held in place by a rubber band. When the plant had reached the desired age, female leafhoppers known to be ovipositing were introduced through a round opening in the side of the cylinder at the rate of 2 insects per fully spread leaf and 1 additional for the terminal growth.

The caged plants were set up in a laboratory that measured about 8 m x 10 m and had two wide bay-windows opening to the north. Temperature was maintained fairly constant at about 25°C and the relative humidity fluctuated between 50 and 70%. Four 2-tube, 24-ft fluorescent ceiling lamps provided abundant illumination.

The plants were observed for 3 days from 5:00 a.m. to 6:00 p.m. and at irregular intervals during the evening up to 11:00 p.m. The insects seemed to spend the major part of their time on the underside of leaves but were also seen for variable periods on the leaf petioles and on the stem. They were not observed to be particularly attracted to the succulent terminal shoots and showed preference for the trifoliate leaves only if these were fully expanded. Following exposure to the

insects, the plants were cut into sections and cleared in hot lacto-phenol according to the method perfected by O. V. Carlson and E. T. Hibbs (1962). The eggs were counted and averaged for each plant section. A similar procedure was used for lima bean, but only of one age, namely 15 days. The results are represented graphically in Fig. 24 and summarized below in Table 3.

Table 3. Number of eggs per section of cowpea (Vigna sinensis) and lima bean (Phaseolus lunatis)

Plant sections	Cowpea			Lima bean
	12 days	15 day	24 day	15 days
1. Stem below simple leaves	1	0	0	0
2. Petioles of simple leaves	22	9	4	4
3. First internode	31	3	0	0
4. Petiole of first trifoliate	-	31	20	4
5. Second internode	-	6	0	0
6. Petiole of second trifoliate	-	3	21	9
7. Median leaflet of first trifoliate	-	-	-	6
8. Third internode	-	-	0	-
9. Petiole of third trifoliate	-	-	38	-
10. Fourth internode	-	-	0	-
11. Petiole of fourth trifoliate	-	-	3	-
12. Terminal growth	-	-	29	-

In summary

1. The placement of eggs is shifted in an upward direction as the plant grows and matures.
2. No eggs were found within leaf veins of cowpea or lima bean.
3. Youthful (terminal) growth is not sought after for egg deposition.

4. Leaf petioles are a choice site, regardless of leaf age, except for the very early or very late stages of leaf development
5. The terminal growth becomes a significant oviposition site late in the development of the foliage after the lower parts of the plant have aged

In lima bean, the leaflets are attached by petiolules to a common petiole or rachis. This feature was expected to inform further on the localized character of the oviposition site. Indeed, the eggs were observed clustered in the central petiolule.

The favorite oviposition site was thus determined and subsequent observations could be made about the development cycle of ! phaseola. The snapbox technique perfected by O'Keeffe (1965) was used extensively.

Rate and Duration of Oviposition

The total number of eggs laid by mated females was determined by maintaining the insects on excised petioles within $3.5 \times 3.5 \times 3.5$ cm clear polystyrene plastic boxes. A lateral aperture 0.8 cm in diam. was provided for the introduction of the test insect. The plant material was renewed every 2 $\frac{1}{2}$ to 3 days and the cages cleaned of honeydew. Eggs were counted in petioles cleared in hot lactophenol at each period of petiole change until the female died. The time lapse between consecutive petiole changes was precisely recorded so that the oviposition rate per 24 hours could be calculated.

In order to determine whether the type of food might have an influence on either the total number of eggs deposited or the duration of

egg deposition, the three leguminous host plants were sequentially used with approximately 60 specimens in each case. No special consideration was given to the stage of maturity of the plant material. Only mated females were included. Some mated female would not oviposit and were discarded. The data recorded in this exploratory trial are condensed in Table 4.

Table 4. Average total number of eggs per female and duration of oviposition of P. phaseoli on three leguminous hosts

	<u>Phaseolus</u> <u>vulgaris</u>	<u>Phaseolus</u> <u>lunatus</u>	<u>Vigna</u> <u>sinensis</u>
Total eggs laid per female	159.23	115.97	32.43
Duration of oviposition (days)	74.97	64.97	61.12

Egg development

Plants with fertilized eggs in their tissues were removed from the oviposition cages and the presence of females to controlled conditions of a growth chamber. The plants were examined at frequent intervals especially in the morning. The date and hour of emergence were noted and the first instar nymphs were removed with a fine camel's-hair brush (washed in distilled water) as soon as they could be seen and sent toward the neighboring leaf. The average incubation period was between 8 and 10 days.

Oviposition by Virgin Females

All excised petioles from all cages were processed at each change and searched for eggs. This led to the finding that some females which had not been observed copulating were, nevertheless, depositing eggs.

In order to establish whether or not virgin females deposited viable eggs, fifth instar nymphs were collected from one of the leaf-hopper colonies on common bean and introduced individually into cages with bean petioles. At emergence, the males were discarded and the females maintained encaged through 4 changes of petioles, (i.e. about 12 days). The petioles at each change were cleared in lactophenol and examined for eggs. The numbers of eggs which were counted from 20 such virgin females are tabulated in Table 5. These ovipositing, non-mated females were then placed on potted young bean plants which were free of infestation. They were allowed to stay 6 days and were then liberated. The plants, now without cages, were introduced in controlled environment chambers and observed for emergence of nymphs (fig. 16).

After 7 days, and later after 10 days, 3 plants were selected at random, and the sections processed in lactophenol and searched for developing eggs. The eggs from the virgin females showed no definite outline or content, instead, oblong cavities, slightly tan-colored, were observed suggesting the emplacement of eggs which had failed to develop and had decayed.

Table 5 Oviposition by virgin females

Female number	Periods during which eggs were counted (3 days)			
	1	2	3	4
1	0	0	0	6
2	0	0	0	0
3	0	0	7	7
4	2	7	- ^a	- ^a
5	5	7	- ^a	- ^a
6	0	5	- ^a	- ^a
7	7	9	- ^a	- ^a
8	0	0	8	4
9	0	0	3	9
10	0	0	8	4
11	0	5	0	0
12	0	0	4	1
13	6	11	0	0
14	0	0	3	6
15	0	0	0	0
16	0	0	6	0
17	0	0	9	6
18	0	0	7	8
19	0	0	0	0

^aegg count omitted by error

Nymphal Development

All nymphs born in the course of one day were promptly transferred to rearing cages 3.5 x 3.5 x 3.5 cm hinged plastic boxes attached by way of rubber tubing to florist's waterpicks filled with 3% sucrose solution. Petioles with petiolules and a small section of leaf area were used as food substrate. The portion of foliar surface was intended to facilitate the feeding of newly born nymphs and offer them a shaded site, since at that stage of their development leafhoppers display strong negative phototropism.

All cages were numbered and examined twice daily at 6:00 a.m. and 6:00 p.m. The duration of the different instars was determined by the number of exuviae. The shed skins were counted at each observation and each additional one meant the passage from one instar to the next (up to a maximum of 4), at which time the adult emerged. The days were divided into two halves of 12 hours each, the moultings (if any) occurring during the night or early morning falling into the first half and those occurring during the day into the second half. The time of emergence of the adult was recorded and the sex determined.

All three legumes were tried as food substrates mostly to determine whether they would present any problems and could be safely included in planned experiments. Table 6 summarizes the data which were thus obtained and they are expressed both as individual instars and total average length of the development cycle. The given data represent averages over 38, 42, and 31 specimens raised respectively for at least 10 consecutive generations on common bean, lima bean, and cowpea.

Table 6 Average duration (in days) of the nymphal stage of L. phaseola reared on three leguminous hosts

Host plants	Instars					Total duration
	I	II	III	IV	V	
<u>Phaseolus vulgaris</u>	2 20	1 87	1 97	2 04	3 61	11 66
<u>Phaseolus lunatus</u>	2 51	1 84	2 04	2 39	3 85	12 60
<u>Vigna sinensis</u>	3 31	2 13	2 37	2 53	3 84	14 19
Means	2 67	1 95	2 13	2 32	3 77	12 84

Sex Ratio

Among the 111 individuals which successfully completed their development on the three leguminous hosts, the sexes were found to be distributed as follows

Table 7 Sex ratio of L. phaseola reared on three leguminous hosts

Plant host	Females	Males	Totals	Male Female sex ratio
<u>Phaseolus vulgaris</u>	18	20	38	1 07
<u>Phaseolus lunatus</u>	20	22	42	1 10
<u>Vigna sinensis</u>	15	16	31	1 12
TOTALS	53	58	111	1 09

The overall sex ratio was thus found to be 1 09, indicating a very slight predominance of male births

Longevity of Mated Females

The life-span of inseminated females of P. phaseola was evaluated on all three substrate types. The observation cages attached to water-picks in the usual manner were of slightly larger dimensions, 4.5 x 5.5 x 3.5 cm, to accommodate a longer section of petiole and median petiolule. The food was renewed at suitable intervals and the snapboxes freed of honeydew. This cleaning was found to be increasingly important as the specimens aged and weakened. They would frequently be observed with the wings glued to the sugary exudate, trying to free themselves. The specimens found dead, immobilized by honeydew, were discarded from the computations. Table 8 summarizes longevity figures averaged on 37 mated females reared on common bean, 38 on lima bean, and 34 on cowpea.

Table 8 Longevity of mated females of P. phaseola on three leguminous hosts

Host plants	Average number of days lived
<u>Phaseolus vulgaris</u>	76.75
<u>Phaseolus lunatus</u>	62.89
<u>Vigna sinensis</u>	61.62
Overall mean longevity	67.16

Mating

Another adaptation of O'Keeffe's technique was used in that test. It consisted essentially in maintaining appropriate sections of plant

material in a fresh condition within small observation cages of clear plastic. Leaf petioles were used with the central petiole attached. The sections measured totally about 8 cm and the cut end was immersed in a 3% sucrose solution contained in a florist's waterpick. The observation cage was a plastic snapbox, 4.5 x 5.5 x 3.5 cm, clasped on a piece of plastic tubing 0.9 cm in diameter, about 4 cm long, thrust into the neoprene cap of the waterpick. The snapbox was provided at the side with an additional circular hole for the introduction of test insects.

After it was found that several test insects would be killed accidentally while snapping the cage closed, a small modification was introduced, as seen in Fig. 17. Instead of having the snapbox clasped to the tubing thrust into the waterpick, the piece of tubing was made to fit tightly into a round opening provided on one half of the snapbox. The latter could be opened within a transfer cage (Fig. 23), cleaned of honeydew, or otherwise manipulated without separating cage and waterpick. The insect naturally escaped but was retrieved easily with a small glass tube and returned to the cage now clean. Cleaning of the observation cages and renewal of the plant material were performed together. The transfer cage was 60 cm long, 50 cm wide, 50 cm tall, with sides of fine screen screening and a top of plastic film. The front part was divided into two halves. The upper, fitted with plexiglass, served as an observation window, the lower was furnished with black cloth to be used as a sleeve.

To facilitate recapture, a bright light source was sometimes

maintained opposite the transparent top. When eggs had to be counted, the petioles, before clearing in hot lactophenol, were identified with labels of bond paper strung with No. 10 gauge sewing thread.

In the course of the research, mating pairs were often observed in the screenhouse and in the laboratory. It became promptly evident that copulation was more frequent in the early morning hours. Some matings did occur late in the forenoon, very few were observed in the afternoon and none in the evening. To determine whether copulation would occur during the night, visits were occasionally made to the laboratory, though not as regularly and frequently as during the daytime. The lights were not switched on and inspection was carried out with a flashlight. The insects were found to be extremely quiet and inactive in contradistinction to the great mobility and restlessness which are displayed when matings are frequent.

The mating schedule as mentioned above was not disrupted under conditions of controlled temperature and humidity nor under artificial illumination. The intensity of light did not seem to have any significant effect, as evidenced by comparable activity during bright and cloudy days. Although day lengths in the tropics do not vary appreciably at different times of the year, slight variations do occur and photoperiod in the laboratory was changed to mimic the field conditions. In general terms, an average day is equally divided between daylight and darkness.

A first group of 52 females was observed on common bean (Phaseolus vulgaris L.) About 200 fifth instar nymphs were collected from the

acrim, etc. in the greenhouse and introduced individually in observation cages, $3.5 \times 3.5 \times 3.5$ cm, with lean potatoes as food. Records were kept of the time of emergence of both sexes and of the time that males were introduced into the cages of the virgin females, which were numbered. Two males were provided for each female.

Observations were made at hourly intervals from about 5:30 a.m. to 6:00 p.m. The time and duration of copula were recorded. They are summarized in tables 9, 10 and 11. Once mating was accomplished, the males were promptly removed and the inseminated females were retained uncaged, under the same identifying number, for study of oviposition and longevity. As mentioned earlier, the food was renewed about every 3 days, at which time the cage was cleaned of honeydew with a swab of cotton dipped in distilled water.

Although no particularly long time was spent watching mating behavior, many of the events reported by workers such as O. V. Carlson (1967) were noted, e.g. increase in activity, preening, parallel positioning of the mites, swift circling ending in the interlocking of the genital appendages, tapping of the sides of the male by the female while in copula. Interlocked male and female reacted in unison to brisk disturbances in the environment such as shift in the substrate, sudden change in illumination, strong noises, and movements of surrounding persons or objects. The mating couple would move in harmonious coordination and, as a rule, would not separate, except perhaps, if they had been copulating for considerable time. Pairs accidentally shaken off foliage dropped onto the bottom of the cage or onto the soil and

Table 9 Time and duration of mating of 1 phiscola on common bean
(Pha colus vulgaris)

Beginning observed at	Termination observed at	Total duration (hours)
7 30 a m	9 00 a m	1 hr 30 min
7 10 "	10 15 "	3 " 05 "
6 35 "	8 00 "	1 " 25 "
6 30 "	7 40 "	1 " 10 "
12 45 p m	2 15 "	1 " 30 "
6 35 a m	9 00 "	2 " 25 "
4 30 p m	6 30 p m	2 " 00 "
7 45 a m	9 45 a m	2 " 00 "
6 00 "	8 40 "	2 " 40 "
5 45 "	8 15 "	2 " 30 "
9 20 "	10 00 "	0 " 40 "
5 45 "	7 40 "	1 " 55 "
6 00 "	8 50 "	2 " 50 "
5 45 "	8 20 "	2 " 35 "
8 30 "	10 30 "	2 " 00 "
5 30 "	7 30 "	2 " 00 "
6 30 "	8 00 "	1 " 30 "
6 00 "	8 00 "	2 " 00 "
7 00 "	9 10 "	2 " 10 "
6 00 "	7 45 "	1 " 45 "
8 00 "	10 15 "	2 " 15 "
6 30 "	8 20 "	1 " 50 "
8 10 "	10 30 "	2 " 20 "
7 15 "	9 30 "	2 " 15 "
6 00 "	7 45 "	1 " 45 "
6 00 "	8 30 "	2 " 30 "
9 30 "	10 50 "	1 " 20 "
6 30 "	8 10 "	1 " 40 "
6 00 "	8 30 "	2 " 30 "
8 15 "	10 30 "	2 " 15 "
6 00 "	8 40 "	2 " 40 "
6 00 "	7 25 "	1 " 25 "
10 30 "	12 05 p m	1 " 35 "
6 30 "	8 10 a m	1 " 40 "
5 45 "	7 30 "	1 " 45 "
7 40 "	8 00 "	0 " 20 "
6 00 "	8 40 "	2 " 40 "
Total time		66 " 05 "
Number of pairs		37
Mean mating duration		2 hr 20 min

Table 10. Time and duration of mating of F. phascolia on lima bean
(Phaseolus lunatus)

Beginning observed at	Termination observed at	total duration (hours)
6 30 a m	9 00 a m	2 hr 30 min
5 10 "	7 20 "	2 " 10 "
5 25 "	7 45 "	2 " 20 "
6 00 "	8 00 "	2 " 00 "
5 35 "	6 45 "	1 " 10 "
5 20 "	7 45 "	2 " 25 "
5 30 "	7 45 "	2 " 15 "
5 30 "	6 40 "	1 " 10 "
7 00 " a	9 00 "	2 " 00 "a
6 15 "	7 20 "	1 " 05 "
9 30 "	10 40 "	1 " 10 "
5 30 "	8 00 "	2 " 30 "
6 00 "	7 40 "	1 " 40 "
7 15 "	8 15 "	1 " 00 "
7 00 "	8 30 "	1 " 30 "
6 00 "	8 40 "	2 " 40 "
5 45 "	8 00 "	2 " 15 "
7 30 "	8 50 "	1 " 20 "
5 30 "	7 30 "	2 " 00 "
5 15 "	7 30 "	2 " 15 "
3 40 p m	4 45 p m	1 " 05 "
9 00 a m.	11 00 a m	2 " 00 "
7 20 "	8 35 "	1 " 15 "
5 15 "	8 00 "	2 " 45 "
5 20 "	8 00 "	2 " 40 "
5 45 "	7 15 "	1 " 30 "
10 45 "	12 45 p m	2 " 00 "
5 15 "	6 45 a m	1 " 30 "
9 45 "	11 00 "	1 " 15 "
5 15 "	7 15 "	2 " 00 "
5 20 "	7 00 "	1 " 40 "
7 00 "	8 30 "	1 " 30 "
8 00 "	9 25 "	1 " 25 "
5 15 "	7 30 "	2 " 15 "
1 30 p.m	2 30 p m	1 " 00 "
6 00 a m	7 45 a m	1 " 45 "
7 00 "	8 30 "	1 " 30 "
1 00 p.m	2 30 p m	1 " 30 "

^aSuspected to have started earlier

Table 10 (continued)

Beginning observed at	Termination observed at	Total duration (hours)
5 30 a m	6 30 a m	1 hr 00 min
7 00 "	8 30 "	1 " 30 "
6 00 "	7 30 "	1 " 30 "
6 45 "	8 50 "	2 " 05 "
5 30 "	7 30 "	2 " 00 "
	Total time	71 " 35 "
	Number of pairs	43
	Mean mating duration	2 hr 05 min

Table 11. Time and duration of mating of 1 phoca on cow per
(Vulpes sinensis)

Beginning observed at	Termination observed at	Total duration (hours)
7 00 a m ^a	9 15 a m	2 hr 15 min ^a
9 25 "	12 00 "	2 " 35 "
7 00 " a	9 45 "	2 " 45 " a
10 00 "	11 40 "	1 " 40 "
8 35 "	10 30 "	1 " 55 "
6 10 "	8 00 "	1 " 50 "
6 15 "	8 00 "	1 " 45 "
7 00 " a	8 25 "	1 " 25 " a
7 10 " a	9 45 "	2 " 35 " a
9 20 "	11 15 "	1 " 55 "
8 30 "	10 35 "	2 " 05 "
7 30 "	10 45 "	3 " 15 "
6 00 "	8 50 "	2 " 50 "
7 20 " a	10 40 "	3 " 20 " a
7 00 " a	8 30 "	1 " 30 " a
7 00 " a	9 15 "	2 " 15 " a
6 20 " a	7 50 "	1 " 30 " a
7 30 "	11 45 "	4 " 15 "
6 15 "	8 00 "	1 " 45 "
8 30 "	11 15 "	2 " 45 "
7 50 "	9 15 "	1 " 25 "
7 00 " a	9 35 "	2 " 35 " a
7 10 "	11 10 "	4 " 00 "
9 50 "	12 30 "	2 " 40 "
6 35 "	8 00 "	1 " 25 "
8 50 "	10 30 "	1 " 40 "
7 00 " a	10 30 "	3 " 30 " a
7 00 " a	10 00 "	3 " 00 " a
8 30 "	11 30 "	3 " 00 "
7 00 "	9 00 "	2 " 00 "
7 00 " a	9 00 "	2 " 00 " a
Total time		67 " 05 "
Number of pairs		31
Mean mating duration		2 hr 16 min

^a Suspected to have started earlier

remained coupled. In general, however, all possible causes of disturbance were carefully avoided, especially at the times when matings were at a peak, between 5 00 a m and 11 00 a m.

In addition to information about time and duration of mating, the ages of both sexes at mating were also recorded and are summarized in Table 12. In order to bring out such information as length of premating in either sex, relative importance of male and female ages in determination of mating, interaction of male and female ages, the mating data were organized as follows:

- a Age of female at exposure to males
- b Age of males at exposure to female
- c Time lapse between exposure of sexes to each other and mating occurrence
- d Age of female at mating
- e Age of the males at mating (it was not difficult to use males of the same age, that is, born the same day)

A statistical analysis was made of these data and is presented within the experimentation section.

Feeding of Empoasca leafhoppers on Legumes

The great majority of bean fields in Central America are invariably invaded by Empoasca leafhoppers. Many wild species appear also to be particularly sought after by the insects. Generally, two to three bean plantings are realized in one year. The first is initiated as soon as the "spring" rains have started in April and is harvested by late June to mid-July,

Table 12. Mating age (in days) of L. phaseola on Phaeocephalus vulgaris

Age of female at exposure to males	Age of males at exposure to female	Time lapse be- tween exposure and mating	Age of female at mating	Age of males at mating
2	0	7	9	7
0	0	12	12	12
0	0	7	7	7
5	0	6	11	6
6	35	12	18	47
0	0	6	6	6
1	1	3	4	4
5	6	3	8	9
0	14	6	6	20
6	0	3	9	3
2	9	4	6	13
5	7	1	6	8
3	21	7	10	28
6	0	5	11	5
3	0	5	8	5
2	0	2	4	2
4	5	2	6	7
2	0	7	9	7
1	1	8	9	9
1	1	8	9	9
6	1	5	11	6
1	1	9	10	10
6	0	6	12	6
0	11	5	5	16
1	21	10	11	31
8	0	4	12	4
2	0	7	9	7
2	0	5	7	5
2	5	12	14	17
9	7	0	9	7
2	4	4	6	8
4	8	2	6	10
0	10	27	27	37
0	8	12	12	20
0	0	9	9	9

in dry weather. The second is established in early August and is mature in November, when the second rainy season is over. Impoacan leafhoppers build up during the first crop and transfer in great numbers to the second, jeopardizing, or annihilating all production of commercial beans. Most often, the plantations will not fail altogether but will be diversely affected depending on the intensity of the invading population. When the pest density is very high, the bean field will quickly be damaged beyond recovery and the leafhoppers will disperse to other food plants.

An infestation of moderate intensity will retard axillary growth and bring about a profusion of axillary branching which largely compensates for the arrested terminal elongation. If the infestation persists, stunting of new growth, crinkling and roughening of the foliage will develop. After some time, the foliage will start to yellow. In the sequence of symptoms, yellowing is usually the one which raises alarm in the farmer. Strangely, the other damage expressions, though of greater physiological consequence, often escape notice or are not associated with the presence of the insects. In the areas where L. phaseoli predominated, damage expression was less striking, though essentially the same is for kraemeri, and was given even lesser consideration.

The plant reaction to leafhopper attack was observed to vary in different types of legumes. Common bean (Phaseolus vulgaris L.) showed great sensitivity to the insect attack, reacted with abundant axillary growth, encouraging further proliferation of the pest, and succumbed

rapidly to its damage. In the screenhouse, the cages had to be infested with relatively few insects so that the abundant progeny would find food enough to complete its development. Lima bean (Phaseolus lunatus) fostered numerical increase to a much lesser extent. If the invading population is strong, it will seldom succeed in killing the plants, instead, that population will dwindle and will sometimes come close to extinction. The plants are heavily damaged but will recuperate, producing new foliage for the progeny and the few survivors (Fig. 10). Cowpea (Vigna sinensis) will tolerate high numbers of insects without apparent damage, axillary compensation is slow and of little significance. Under field conditions, nymphs are seldom seen on the latter species, indicating that it is not readily infested.

The pattern, as well as the extent of yellowing were observed to be different with the two predominant species of Cicadellidae (Figs 18 and 19). L. kræmeri damage, in addition to developing faster, showed a pattern which recalls hopperburn, i. e. a marginal drying up of the tissues, downward (instead of upward) curling of the foliar edge. E. phaseola damage is slower to develop and displays a distinct angular yellowing. In contrast to the damage inflicted by L. kræmeri (Fig. 19, lower series of 3 leaves), the damage by E. phaseola may never extend to the entire marginal area of the leaf (Fig. 19, upper 3 leaves) unless necrosis sets in, completely destroying the leaf (Fig. 18, upper left corner). Fig. 18 shows in a clockwise order, the progressive changes which took place in the bean leaf as a result of damage by E. phaseola, the healthy leaf occupying the center of the illustration.

from the preceding observations and exploratory trials it was obvious that the feeding and oviposition of E. phaseola were far from being indiscriminate and that the quality and type of the substrate had to be taken into account if the biological processes of the leafhopper were to be assessed with some degree of precision.

The observations and trials carried out indicated plainly that E. phaseola displays different rates of oviposition and longevity on different host plants. One plant species will foster the reproduction and lengthen the life of the insect and others will prove to be less favorable substrates. None of the three legumes included in the tests interfered drastically enough with the biology of the leafhopper to suggest potent inhibitory qualities, though inhibitors may occur in other strains. It has already been mentioned that the observed lack of attractiveness of cowpea to leafhoppers in the field is overcome in confinement. It would be of great interest to determine what plant quality is the basis for the insects' limited acceptance.

EXPERIMENTATION

Two experiments were conducted to measure the influence of the host plant upon fecundity and longevity of L. phaseola

Experiment I

The object of this experiment was to measure and compare the rate of egg deposition and its duration among females restricted to common bean plants of pre-bloom and post-bloom maturity and to observe whether or not the oviposition rate would be influenced (and how quickly influenced) if ovipositing females were transferred after 30 days on petioles of post-bloom plants to the petioles of pre-bloom plants

Materials and methods

Greenhouse plantings of common bean were timed to provide abundant plants of pre-bloom and post-bloom maturity during the course of the experiment (figs 14 and 15)

Erlenmeyer flasks (500 cc), filled with 3% sucrose solution, were fitted with cork stoppers in which 4 holes were perforated. Four leaves clipped from the median portion of mature plants, about 30 days from planting, were cautiously introduced through the holes in the stoppers and the cut ends of the petioles immersed in the sugar solution (Fig 20). The leaf blades were oriented as far apart as possible to avoid overshadowing and to provide aeration and freedom of movement for the insects engaged in feeding and mating. The flasks were scattered over the available bottom surface of a transfer cage (fig 12) which was lighted with a 2-tube fluorescent lamp set close to the transparent plastic top.

The test insects were taken from several rearing cages in which leafhoppers had been continuously raised on the same host for at least 8 to 10 generations. They were captured as fifth instar nymphs and taken to the laboratory, where about 500 of the nymphs were released gently and uniformly over the foliage in a transfer cage. Here they were held for 9 days, 2 for the completion of the last instar and 7 for premating. The cage was frequently observed for emergence to the adult form and possible mating. Pairs in copulo started to show in appreciable number around the 7th day and continued through the 8th and 9th. Early on this last day the sexes were separated and the females individually caged.

Groups of 50 individually caged females were distributed randomly among the following treatments:

- A Oviposition cage continuously supplied with petioles of plants in pre-bloom stage
- B Oviposition cage supplied with petioles of post-bloom plants for 30 days, then transferred to pre-bloom petioles
- C Oviposition cage continuously supplied with post-bloom petioles

The oviposition cages were clear plastic snapboxes 3.5 x 3.5 x 3.5 cm with three holes, one slightly larger than an average petiole (about 4 mm in diam), a second on the opposite side wide enough to receive a piece of plastic tubing 7 mm in diam, and a last one on the lateral side of the hinged half of the snapbox, about 5 mm in diam.

The excised leaf was inserted first through the small upper hole, then cautiously pushed across the section of tubing, the free end immersed in a florist's waterpick filled with a 3% sucrose solution (Fig. 22). Before the cages were closed, cotton was gently caulked around the petiole at the apertures to prevent escape of the female and protect the plant part against rupture or bruises. It was soon seen preferable to first wrap the petioles in wetted cotton prior to their insertion in the snapboxes at a height corresponding to the upper hole, perfecting the caulking later with a pointed instrument. It was easier and less hazardous to install the cotton plug around the base of the petiole in the section of plastic tubing. The cages were then ready to receive the females which were introduced through the lateral hole, which was closed with a cotton plug.

Leaves from the sequential plantings in the greenhouse were collected to supply the oviposition cages in the following manner. Approximately 160 leaves, from pre-bloom plants (27 days from planting), or from post-bloom plants (39 days from planting), were clipped close to their junction with the stem, placed to stand in water, and promptly taken to a cool and well illuminated room. To accomplish this with the least damage to the plant material, shallow channel pans were used. Rectangular sections of 4-in. mesh hardware cloth were cut and mounted on wooden frames to fit into the pans and to come up flush with the containers' brim. The pans were filled with water so that the leaves' petioles would be immersed while the blades rested flat on the screen surface.

At the time of foliage renewal and egg count, the petioles were clipped at the upper surface of the cage, pulled gently 2 to 3 cm out of the plastic tube and again clipped. Pulling out the petiole a short distance was necessary because, often, the insect would oviposit very close to the petiole base or even descend within the tube to deposit eggs. The sections thus excised represented rather exactly the length of substrate on which the female had been feeding and ovipositing. The petioles were slit lengthwise with a razor blade, labelled, and cleared in boiling lactophenol for 2½ minutes. The practice was soon adopted to let them stand in cold lactophenol overnight, the egg count to be carried out the following morning.

Records were kept of the number of eggs deposited and the number of days lived. This made possible the assessment of the following biological variables: (1) number of eggs deposited during the insect's entire life as a fertilized female, (2) duration of the oviposition period, (3) daily oviposition rate, (4) overall rate of oviposition for the total period of egg deposition, and (5) the total life-span.

The daily oviposition rate was determined for each female by dividing the number of eggs deposited in the interval of two consecutive leaf changes by the number of hours that interval lasted and multiplying the quotient by 24. The figures thus obtained were averaged for the number of females alive during the interval being considered.

The curves obtained by plotting the daily oviposition rates against the days of petiole renewal were tempered at first by the relatively large sample size, but became more and more sensitive as the number of surviving females became smaller.

to get away from this bias, an over-all ratio was computed for each individual, dividing the total number of eggs deposited by the total number of days lived. By plotting the values thus obtained, a distribution could be determined, this time on the basis of an over-all level of oviposition (Figs. 26, 27, and 28). Any clustering of point at one age-level would be indicative of the relatively critical importance of this particular period of the insect on fecundity.

Results

Total number of eggs deposited The effect of treatments A, B, and C on the number of eggs deposited was studied by comparing treatment A to B, treatment A to C, and treatment B to C. For each treatment the mean and the variance were calculated so that t values could be determined for all 3 comparisons mentioned above, as follows:

$$t_{AB} = \frac{\bar{X}_A - \bar{X}_B}{\sqrt{\frac{s_A^2}{n_A} + \frac{s_B^2}{n_B}}} = \frac{176.41 - 153.78}{\sqrt{\frac{15,555.62}{46} + \frac{12,776.06}{46}}} = \frac{22.63}{\sqrt{615.90}} = 0.91$$

$$t_{AC} = \frac{\bar{X}_C - \bar{X}_A}{\sqrt{\frac{s_C^2}{n_C} + \frac{s_A^2}{n_A}}} = \frac{43.39}{\sqrt{544.33}} = 2.17$$

$$t_{BC} = \frac{\bar{X}_B - \bar{X}_C}{\sqrt{\frac{s_B^2}{n_B} + \frac{s_C^2}{n_C}}} = \frac{26.76}{\sqrt{483.91}} = 1.21$$

The number of females was 46 in each of the 3 treatments, the number of degrees of freedom per comparison was $(46-1) + (46-1) = 90$. The corresponding tabulated t values were respectively 1.44 and 2.66 as interpolated from the values for 60 and 120 d f, only comparison AC was significant and only at the 5% level.

Duration of oviposition the t values calculated for AB, AC and BC were -0.12, -0.35 and -0.22, respectively. None was significant. In other words, the age of the leaves had no effect on the duration of oviposition in all cases considered.

Daily and overall oviposition rates these rates are presented in Figs. 25 to 28.

Fig. 25. The lines representing the 3 treatments are all broken, to a greater or lesser extent. No correspondence could be found in the time of occurrence, the frequency or the amplitude of peaks and troughs in the curves. Some features, however, could be observed in all three:

1. An initial steep drop,
2. A gradual return to the original rate level,
3. A steady decrease from the point mentioned under 2 to

the termination at zero rate.

A particularly sharp peak at period 17 in the curve describing treatment A stands out. Time was taken to investigate this apparent abnormality. The individual curves for the females involved in the averaged rate were plotted on the same coordinates and the outstanding peak value was found to represent the correct mean for the values. Reasons for this occurrence will be proposed in the discussion.

Fig. 26 (Treatment A) The majority of the points are located at about a level of 3 and only a few at or near zero. A rather large clumping is present at 120 days. The young plant material would seem to maintain a high oviposition rate throughout the life of the females.

Fig. 27 (Treatment B) The points are scattered and average at about a level of 2.5. There is no clustering at a late age and the duration of oviposition is extended. The number of low values is relatively small. An increase in rate is visible after 30 days, following the transfer from post-bloom to pre-bloom.

Fig. 28 (Treatment C) The rates fall along a line which approximates a level of about 2 and are therefore slightly lower than in the preceding treatments. There can also be observed a greater number of low values between 0 and 1.

Life-span The longevity means were compared giving the following t values: AB = 1.43, AC = 2.36*, BC = 0.27. Referring again to the tabulated values for 90 d.f. given previously, only AC was significant and only at the 5% level. So, a significant difference in longevity was found only between the females maintained throughout the experiment on either pre-bloom or post-bloom petioles, the change from old to young material showed no significant effect upon longevity.

Experiment II

A second experiment was designed to expose the effect of selected host plants upon the rate and duration of oviposition and as well the longevity of the female leafhoppers. Of the plant species included in

exploratory trials, two legumes, the common bean and the cowpea, were selected. Fecund female leafhoppers raised continuously on each of the two host plants were maintained on the same host or abruptly transferred from one host to the other and the effects noted.

Materials and Methods

Both the common bean and cowpea were easy to grow in the screen-house and produced foliage in adequate quantity of uniform maturity for implementing the simultaneous treatments.

Plant material of intermediate age (30 days from planting) was used in all cases and the median portions of plants exclusively utilized, avoiding both youthful and decadent foliage. It was assumed that differential levels of physiological activity in the plant, as affected by age, could be minimized if the same degree of plant maturity were provided in all treatments. Petioles and the attached median petiolule, were used in the tests.

These petioles were placed in florist's waterpicks, the lower cut end immersed in 3% sucrose solution. These waterpicks, in turn, were attached (as in Experiment I) by way of a small section of plastic tubing to the feeding and oviposition cages, which were clear plastic snapboxes 5 x 5 x 5 cm. These snapboxes were perforated in the center of the bottom side so that the 0.7 cm hole would be equally divided between the two hinged halves, the second hole was bored on one of the two lateral sides.

Approximately 500 fifth instar leafhopper nymphs were collected from screenhouse colonies which had been maintained successfully for

about 10 generations. Half the number of immature insects were taken from common bean and half from cowpea. The insects from each source were placed in separate breeding cages, where each group was grown to the adult stage and permitted to mate in common.

Individual female leafhoppers were removed from the breeding cages to the appropriate oviposition cages in groups of 50 per test, each oviposition cage containing a leaf petiole of the appropriate test plant to accomplish the following five treatments:

- I Reared on cowpea, tested on cowpea ($V \rightarrow V$)
- II Reared on cowpea, tested on common bean ($V \rightarrow P$)
- III Reared on common bean, tested on cowpea ($P \rightarrow V$)
- IV Reared on common bean, tested on common bean ($P \rightarrow P$)
- V Reared on common bean, tested for 30 days on cowpea, returned to common bean, ($P \rightarrow V \rightarrow P$)

The petioles and the sucrose solution were renewed every $2\frac{1}{2}$ to 3 days and the cages cleaned to remove honeydew at each time of change. The plastic oviposition cages were opened within a transfer chamber (Fig. 23) and the petioles gently pulled out of the plastic tubing about 1 to 2 cm. They were then clipped, slit lengthwise to a depth about half their thickness with a razor blade, and labelled. Clearing was carried out in boiling lactophenol for 3 minutes. To avoid mixing, the petioles from each treatment were treated separately and kept immersed in cold lactophenol in labelled beakers.

Record were kept of the number of eggs inserted in the tissues up to the time of death of individual females. These data allowed the

determination, for each treatment, of the following data (1) number of eggs deposited, (2) duration of oviposition, (3) overall oviposition rate, (4) daily rate of oviposition, and (5) number of days lived.

The analyses presented hereinafter are comparisons of means of 5 samples of unequal sizes but common population variance. The observations are not paired. In order that the statistical treatment be exposed briefly and more clearly, it was thought that symbols could be assigned to the 5 treatments $X_1, X_2, \dots, X_5$. The means would then be $\bar{X}_1, \bar{X}_2, \dots, \bar{X}_5$, the variances $s_1^2, s_2^2, \dots, s_5^2$.

The effect of the rearing host plant, thus, was determined by coupling the means in the following manner $(\bar{X}_4 - \bar{X}_1) + (\bar{X}_3 - \bar{X}_2)$ or $[(P \rightarrow V) - (V \rightarrow V) + (P \rightarrow P) - (V \rightarrow P)]$, the rearing host plant varies while the experimental host plant does not.

The t value of this effect was calculated, as follows

$$\frac{(\bar{X}_4 - \bar{X}_1) + (\bar{X}_3 - \bar{X}_2)}{\sqrt{\frac{s_4^2}{n_4} + \frac{s_1^2}{n_1} + \frac{s_3^2}{n_3} + \frac{s_2^2}{n_2}}}$$

The variances were obtained from equations such as shown below for

X_1

$$s_1^2 = \frac{\sum X_1^2 - (\sum X_1)^2 / n_1}{n_1 - 1}$$

the number of degrees of freedom was $(n_4 - 1) + (n_1 - 1) + (n_3 - 1)$

$$(n_2 - 1) = 34 + 49 + 49 + 49 = 181$$

Similarly, the effect of the experimental host plant was determined by a t value calculated as follows.

$$t = \frac{(\bar{x}_2 - \bar{x}_1) + (\bar{x}_4 - \bar{x}_3)}{\sqrt{\frac{s_2^2}{n_2} + \frac{s_1^2}{n_1} + \frac{s_4^2}{n_4} + \frac{s_3^2}{n_3}}}, \text{ also for 181 d f, the}$$

interaction between rearing and experimental host plant, as follows

$$t = \frac{\bar{x}_1 + \bar{x}_3 - \bar{x}_4 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_3^2}{n_3} + \frac{s_4^2}{n_4} + \frac{s_2^2}{n_2}}} \quad \text{also for 181 d f}$$

A t value was computed separately for comparison IV to V

$$t = \frac{\bar{x}_5 - \bar{x}_4}{\sqrt{\frac{s_5^2}{n_5} + \frac{s_4^2}{n_4}}} \quad \text{The number of d f in that case}$$

was $(n_5 - 1) + (n_4 - 1) = 14 + 34 = 48$

Results

total number of eggs deposited The procedure outlined above was applied at four different ages of the test insects to detect the possible effect of age and also to bring out more strikingly the effect of re-
verting, from the experimental host plant, back to the rearing host plant. The ages chosen were 15, 45, 75, and 120 days from mating. The calculated t are exposed in Table 13.

Table 13 t values for the effects of rearing and experimental host plant, interaction between the two, and reversion from experimental to rearing host on oviposition of F. phaeola, using common bean (Phaseolus vulgaris) and cowpea (Vigna sinensis)

Variable	Student-t calculated at day			
	15	45	75	120
Rearing host plant	1.48	0.24	0.29	0.26
Experimental host plant	10.09**	15.40*	21.60**	13.59*
Interaction between rearing and experimental host plant	1.17	0.21	0.38	0.19
Reversion from experimental to rearing host plant	0.21	0.12	5.04**	6.31

Significant at 1% level of probability

The tabulated values at the 5% and 1% levels for 181 d.f., were, respectively, 2.16 and 2.77. The t values for the rearing host plant failed altogether to show significance, while those of the experimental host plant were very highly significant. The interaction was, in no case, significant and the reversion to the rearing host from the experimental host proved highly significant following the reversion, which was made on the 21st petiole change, i.e. roughly 60 days from mating.

Duration of oviposition Table 14 summarizes the t values obtained for duration of oviposition in the 5 treatments, the tabulated

values at the 5% and 1% levels are given for 181 (α in the table) and 48 (extrapolated between 45 and 50)

Table 14 Differential effects of rearing and experimental host plants (Phaseolus vulgaris and Vigna sinensis) on duration of oviposition of t. phaseola

Variable	Calculated t values	d f	Tabulated t values 5%	1%
Rearing host plant	2.08	181	2.16	2.77
Experimental host plant	9.48 [*]	181	2.16	2.77
Interaction of rearing and experimental host plant	0.83	181	2.16	2.77
Reversion to rearing host plant from experimental host plant	39.62 [*]	48	1.97	2.68

* Significant at the 1% level of probability

The effects of experimental host plant and of reversion to rearing host were highly significant, while the other effects failed to reach significance.

Daily and overall oviposition rates The daily rate of oviposition is presented synoptically for Treatments I, II, III, IV, and V in fig. 29 and the overall rates for these treatments and Treatment V in figs. 30 to 34

Fig. 29 All 4 curves have a broken course, occasionally showing a peak of unusual amplitude. The common feature is a gradual downward trend. Depending on the host plant used in rearing, there can be an initial drop (more or less pronounced, e.g. in $P \rightarrow V$ and $P \rightarrow P$), no sharp drop as in $V \rightarrow V$, or a rise as in $V \rightarrow P$.

Fig. 30 ($V \rightarrow V$) The points are clustered from 0 to 40 days and spread thinly from 40 to a little over 145 days. The level of egg deposition is generally low with a maximum of about 4.

Fig. 31 ($V \rightarrow P$) The clustering of rather moderately high oviposition rates between 0 and 40 days is not apparent here as in $V \rightarrow V$. The overall rate is maintained at 4 or, in some cases, is higher than that rate. Two females oviposited beyond 140 days, although at very low levels.

Fig. 32 ($P \rightarrow P$) The points are almost evenly distributed between the relatively high rates of 6 and 2. In all cases the rate was then high, but oviposition was comparatively short as compared to $V \rightarrow V$ and $V \rightarrow P$.

Fig. 33 ($P \rightarrow V$) This curve is striking in that all the points are clustered between 0 and a little over 40 days and show a very steep decrease from over 14 down to near 0.

Fig. 34 ($P \rightarrow V \rightarrow P$) The same remarkable pattern is again found as in $P \rightarrow V$ in the first part of the curve. Oviposition drops to 0 from near 40 to near 80 days and picks up from there, maintaining itself in the vicinity of a 2 overall rate. The duration of the oviposition period is extended beyond 140 days.

Effect of Male and Female Age on Duration of Premating

A multiple regression analysis made of the male and female mating data in Table 12 resulted in the following analysis of variance

Table 15 Statistical analysis Effect of male and female ages on the duration of premating

Source of variation	d f	S S	M S	F
Regression	3			
Female age	1	178.60	178.60	10.05**
Male age	1	52.88	52.88	2.97
Interaction	1	0.35	0.35	0.02
Error	32	568.92	17.77	

Significant at 1% level of probability

The F value for the effect of female age is highly significant, the tabulated value at the 1% level being 7.50. The male age and the interaction between the ages of the sexes both failed to show significance even at the 5% level. The tabulated value at that level is 4.15.

DISCUSSION AND CONCLUSION

The results of the experiments and the analysis of their data along with the preliminary observations lead to a few rather well-supported conclusions and also raise several interesting possibilities regarding the relationships of L. phaseola with its host plants in Central America.

On the basis of these studies it may be concluded that

- 1 Leguminous plant parts relatively young, but not youthful, are favorably accepted by the leafhopper L. phaseola and have a measurable effect on the number of eggs deposited by that species. If plant material of this quality is fed continuously to females, the duration of the oviposition and the rate of egg deposition will be increased
- 2 Pre-bloom leaves will extend the life-span of the female leafhoppers to a significant degree. Senescent foliage and stems will assure for some time the survival of the insects. In this, the controlled experiments were in complete accord with the preliminary observations, which revealed that decadent vegetation did not foster egg deposition but could assure survival a rather long time after oviposition had ceased
- 3 The common bean had a distinctly favorable effect on egg production, duration of oviposition, oviposition rate, and life-span

Field observations reinforce these findings. Large numbers of nymphs and adults are common in bean fields

Hardly any infestation occurs on cowpea plantings. The fact that cowpea does not harbor leafhoppers under field conditions is probably indicative that it lacks some attractant. This is further corroborated by the fact that this species allows survival and multiplication of leafhoppers under conditions of confinement. In other words, cowpea does not seem to contain any deterrent to leafhopper survival.

4. L. phaseolus is able to survive on a rather diversified series of host plants.

The case of cowpea which is a mediocre to poor host for the leafhoppers in the field is puzzling. This plant species will sustain the insect when the latter is confined on it but will not favor its longevity nor its egg production. It seems logical that the reason may lie in the quality or abundance of some specific food elements. That nutrients may be limiting is suggested by the observation that leafhoppers may survive on nutriment obtained by feeding on the sap of damaged plants, practically devoid of green tissues. Under these circumstances the females do not oviposit and it is suspected that the eggs do not successfully mature. It is probable, however, that the interruption, even in extreme situations, does not reach the condition of oosorption, the follicle cells ceasing to participate in egg formation and the oocyte dying and being reabsorbed. This seems to be indicated by the strikingly

fast resumption of oviposition in females shifted from a deficient to an adequate host plant. Oogenesis and vitellogenesis would drain heavily on the female leafhoppers' reserves of proteins, nucleic acids, lipids, and glycogen. Replenishment would depend upon the quality of the food source.

5. Mating is largely governed by the age of the female leafhopper. The males are ready to copulate as early as 2 days after they have reached the adult stage. The females have to go through a period of maturation of at least 6 days. Interaction between the sexes was found to be insignificant, males and females mating successfully at all ages and giving rise to nymphs which develop normally.

Since the males can mate several times, they can inseminate females not only of their own generation but of any of the overlapping generations in the bean field, a factor of great importance in the maintenance and increase of leafhopper populations.

6. Under the climatic, vegetational, and agricultural conditions prevailing in Costa Rica and throughout Central America, Trioxys phaeocollis Oman, therefore, quite successfully survives and reproduces in large numbers. As long as such crops as common beans and lima beans are cultivated in small home gardens, the damage by the insect will be important but not prohibitive. The situation can change drastically if large-scale

plantations are established, for example, for the purpose of exportation. The past will no more be inconspicuous but will have to be recognized as one of outstanding importance.

EVALUACION DE VARIEDADES DE FRIJOL POR SU RESISTENCIA AL PICUDO DE LA VAINA Apion Godmani (Wang)

J E Mancia 1/

COMPENDIO

En El Salvador, durante la Epoca lluviosa el cultivo del frijol está influenciado por una serie de factores que inciden en los bajos rendimientos unitarios del mismo, pudiendose mencionar entre estos, los daños ocasionados por las plagas, siendo la de mayor importancia el picudo de la Vaina del Frijol Apion godmani. Por la importancia que este insecto tiene como plaga del frijol y por ser el producto de este cultivo básico en la dieta alimenticia de los salvadoreños, desde el año de 1966, para tratar de buscar una solución integrada al problema, se comenzaron a efectuar una serie de trabajos, entre ellos la Evaluación de Variedades de Frijol por su Resistencia al daño ocasionado por Apion godmani. Para tal efecto se introdujeron al país 2 004 variedades de la Colección Mundial del U.S D A. para ser evaluadas. Su evaluación de campo comenzó en agosto de 1966 y terminó en 1971. La siembra de las variedades y líneas de la Colección Mundial estuvo localizada en el Cantón Izcaquihyo, jurisdicción de Atiquizaya, Departamento de Ahuachapán, lugar escogido por la alta incidencia de la plaga mencionada. La Evaluación se efectuó en base al porcentaje de daño ocasionado por el picudo en las vainas, obteniendose como resultado final once variedades resistentes

INTRODUCCION

El frijol, es básico en la dieta alimenticia de los salvadoreños, es el producto que nos aporta la mayor cantidad de proteínas, no obstante la producción actual del país no llena las necesidades de consumo, debido a que los rendimientos unitarios del cultivo son bajos y consecuentemente ocasiona una fuga de divisas al tener que importarse de otros países de Latinoamérica. Los rendimientos bajos del cultivo están influenciados por varios ---

factores entre ellos se puede mencionar los daños ocasionados por las plagas, siendo en nuestro medio la principal durante la época lluviosa, el picudo de la Vaina del Frijol Apion godmani Wagn, el cual en algunas zonas frijoleras ocasiona hasta un 94% de daño, influyendo directamente en la -- producción del cultivo.

En vista de la importancia del cultivo de frijol, como fuente de proteínas - y vitaminas en la dieta alimenticia del pueblo salvadoreño y por la impor-- tancia que la plaga representa como un factor que incide directamente en - la producción, desde el año de 1966 para tratar de buscar una solución in-- tegrada al problema, se comenzaron a efectuar una serie de trabajos, en-- tre ellos, la Evaluación de Variedades de Frijol por su resistencia al daño ocasionado por Apion godmani.

REVISION DE LITERATURA

Los Mecanismos de resistencia Painter (1941), analizando la resistencia de las plantas de los insectos, encontró que es muy útil dividir el fenóme-- no de resistencia en tres componentes o mecanismos

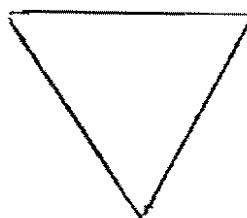
Estos tres mecanismos se enuncian en el diagrama siguiente

PREFERENCIA

Para oviposición
alimento o abri--
go.

ANTIBIOSIS

Efecto adverso de la
planta sobre la bio--
logía del insecto.



TOLERANCIA

Reparación de tejidos, recuperación o habilidad
para soportar infestación de insectos

Preferencia Se usa para definir el grupo de características de las plantas y respuestas de los insectos que, guían o repelen a éstos hacia la utilización de una planta determinada o un grupo de variedades.

Este es un fenómeno complicado, los insectos pueden encontrar sus alimentos guiados por la vista, luz, gravedad y humedad, pero la temperatura y humedad pueden modificar el comportamiento de los insectos y también tienen una influencia diferencial en ciertas variedades, se debe considerar el estímulo mecánico que es un factor aún no bien estudiado

Antibiosis Es la tendencia a prevenir daños o destruir la vida de un insecto. Dethier (1951) la subdivide en dos fases.

a.- Plantas que son deficientes en ambientes requeridos.

b.- Plantas con sustancias tóxicas

La antibiosis parece ser el carácter más deseable en la selección de variedades resistentes en cientos de casos pero en muchas variedades de plantas, otros mecanismos son tan importantes como ésta, sin embargo ningún componente de resistencia es absoluto, casi siempre existen complejas interrelaciones entre diversos factores.

Tolerancia Es un mecanismo de resistencia con el cual la planta muestra una habilidad para crecer y reproducirse, ya sea reparando en parte el daño causado por el insecto, o no mostrando señales de pérdida de vigor a pesar de soportar una población de la plaga aproximadamente igual a aquella, que daña un huésped susceptible (Painter 1951).

La tolerancia es tal vez el mecanismo menos estable en relación con la resistencia, y parece ser el más afectado por las condiciones del ambiente, que actúan en la planta.

Causas de la resistencia Muy importantes conceptos se discuten en el --

libro de Painter (1951) en relación con este tema. Diversos investigadores a menudo encuentran correlaciones entre características morfológicas, -- sustancias químicas, o condiciones fisiológicas de las plantas, cuando analizan los datos obtenidos en comparaciones hechas, con un grupo reducido de variedades, sin embargo, cuando se estudia una colección grande de variedades, las correlaciones no siempre son verdaderas.

Antes de tomar cualquier conclusión sobre la causa de resistencia es necesario tomar en cuenta gran número de factores. Hay tres puntos de evidencia que deben investigarse totalmente, estos son discutidos por Painter - (1951).

- 1 - Evidencia experimental de una íntima relación entre la supues-
ta causa de resistencia y la fisiología o el comportamiento del
insecto
- 2 - Demostrar la completa asociación entre la causa asignada y -
algún factor genético, si es que este factor ocurre, determinan
do esto por medio de la segregación obtenida, de cruzar entre
variedades resistentes y susceptibles.
- 3.- Alta correlación estadística entre la causa asigada y la resis-
tencia.

Wingard (1953) define la resistencia de campo, como el grado
de resistencia suficientemente grande para no permitir a las -
plantas sufrir serios daños, que hagan bajar sus rendimientos

Selección de variedades de frijol resistentes al picudo del ejote Mukelvey
et al, reportan en 1951 algunas observaciones hechas en un grupo de varieda
des resistentes al picudo del ejote en México

Desde 1951 a 1955, se emprendió un trabajo sobre variedades resistentes
al *Apion godmani* Wagner. El método que se usó para seleccionar las varie
dades con cierta resistencia al picudo del ejote se basó en el examen de -

50 vainas colectadas al azar en cada línea o variedad.

Hasta 1950 habían sido seleccionadas las variedades Puebla 32, Hidalgo 6 e Hidalgo 24, para 1951 se aumentó esta lista con las variedades Chiapas 92, Oaxaca 3, Oaxaca 9, Puebla 55, Guerrero 8, Guatemala 33, Guanajuato 20 y Veracruz 10. Todas estas presentaron menos de 0.2 insectos por vaina. En 1952 se examinaron 328 líneas, para seleccionar algunas con resistencia a la plaga y se obtuvo material para trabajos futuros, que variaba desde 0.04 a 0.36 insectos por vaina.

La mayor parte de las selecciones resistentes al picudo del ejote hasta 1954 no tenían gran valor comercial, excepto Rocamex-1 y E A P. 88 B de color amarillo, Hidalgo-12-A-1 y Puebla-2 color negro.

Las variedades de frijol consideradas inmunes en México, pertenecen a la especie Phaseolus multiflorus.

MATERIALES Y METODOS

El ensayo estuvo localizado, en el cantón Izcaquiliyo, Atiquizaya, Departamento de Ahuachapán, escogiéndose este lugar, por ser propio para una buena evaluación del material, debido al gran porcentaje de daño de picudo en las vainas.

El primer paso a seguir fue el de introducir al país 2 004 variedades de frijol, procedentes de la colección Mundial del U S D A.

El trabajo se comenzó en Agosto de 1966, sembrándose surcos de 2 metros de largo por cada variedad y a una distancia de 0.60 cms uno del otro, se fertilizó con fórmula 20-20-0 a razón de 3 qq/Mz.

La evaluación del material se llevó a cabo 15 días antes y al final de su ciclo vegetativo.

La primera evaluación se hizo en cien vainas por variedad, tomando en cuenta únicamente el daño superficial de estas, la segunda se efectuó al --

final del ciclo vegetativo, contando de cada una de las cien vainas, el número total de semillas y el número de estas, dañadas por el picudo, para así sacar un porcentaje de daño y determinar si estas eran inmunes, resistentes, tolerantes o susceptibles a la plaga. También se efectuaron recuentos de picudo por variedad para determinar la presencia de estos, el recuento se hizo directo tomando 10 plantas por surco de 2 mts observando la presencia de Apion en la parte baja, media y alta de la planta, al mismo tiempo anotando daños ocasionados por éstos en las hojas.

Para determinar la resistencia de las variedades, en la primera evaluación, se tomó la siguiente escala

Immune	0 % de daño
Altamente resistente	1 a 5% de daño
Resistente	5 a 10% de daño
Susceptible	10 ó más

RESULTADOS

Como resultados de este trabajo, se obtuvo lo siguiente

- 1.- De las 2004 variedades sembradas para la primera evaluación, se seleccionaron 149 variedades que aparentemente fueron altamente resistentes al picudo de la vaina y 51 variedades resistentes.
- 2 - En la segunda evaluación se seleccionaron 18 variedades que se mostraron altamente resistentes y 73 resistentes, obtenidas de las 200 variedades seleccionadas anteriormente (Ver Tabla 1)
- 3.- De la 3a. evaluación de las variedades llevadas a cabo el 30-XI-68, se obtuvo como resultado 9 variedades altamente resistentes y 2 resistentes
- 4 - En Agosto del 69, se evaluaron nuevamente las once variedades obteniéndose el mismo resultado anterior (Ver Tabla 3)

CONCLUSIONES

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- 1.- Se obtuvieron 9 variedades de frijol con alta resistencia de campo, y dos resistentes al daño ocasionado por el Apion godmani
- 2 - Todas las variedades obtenidas son fotoperiodicas y se pueden sembrar unicamente en Agosto y Diciembre
- 3 - Es necesario hacer una evaluación del material criollo, en busca de resistencia al picudo de la vaina del frijol.

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TABLA 1 Variedades altamente resistentes al picudo de la vaina del frijol Apion godmani (Wagn.) seleccionadas de 200 variedades

13	(Honduras 972)	56	(México 1103)	58	(México 1122)
60	(México 1135)	62	(México 1153)	63	(México 1225)
56	(México 1243)	74	(México 1290)	79	(México 1326)
90	(México 1403)	100	(Nicaragua 1488)	103	(Nicaragua 1495)
105	(Nicaragua 1493)	132	(Canario 1736)	136	(Posa de castilla 1755)
156	(México 140-957 938)	167	(México 1342)	174	(México 1410)

TABLA 2 Variedades altamente resistentes y resistentes al picudo de la vaina.

Nº Entrada	Nombre--Variedad	o/o Daño	AR	S	R
13	972 Honduras	27.5			+
56	1109 México	4.0	+		
58	1122 México	9.5			+
60	1135 México	11.5			+
62	1153 México	2.0	+		
63	1225 México	3.0	+		
66	1243 México	5.0	+		
74	1290 México	3.5	+		
79	1326 México	2.0	+		
90	1403 México	5.0	+		
100	1488 Nicaragua	41.0			+
103	1495 Nicaragua	69.0			+
105 (Testigo)	1499 Nicaragua	89.0			+
132	1736 Canario	57.0			+
138	1755 Posa de Castilla	53.0			+
156	93 - (B) México 150-957	48.0			+
167	1342 Mexico 166	5.0	+		
174	1410 Mexico	6.5			+
154	54-749	5.0	+		

TABLA 3 Evaluación final de variedades de frijol en resistencia de campo al Apion godmani (Wagn.)

Nº Entrada	Nº Picos/10P	Nº Daños 30 hojas	o/o Vainas Dañadas	o/o Daños Semilla	AR	R	S
56	9	126	4.71	2.02	+		
58	16	104	16.00	3.21	+		
62	15	134	4.38	0.87	+		
63	15	81	14.67	7.42		+	
66	15	90	8.52	3.13	+		
74	11	116	12.00	1.46	+		
79	11	68	15.78	4.86	+		
90	17	121	52.72	7.42		+	
154	14	94	5.42	1.30	+		
167	18	87	5.94	1.31	+		
174	11	84	5.24	1.59	+		
105 (T)	20	151	69.25	43.25			+
S A 1 (T)	15	147	99.45	94.07			+

TABLA 4 Reunion de algunas características agronomicas de las variedades alta norte resistentes y resistentes al Apion godmani.

Nº de Entrada	Variedad	Días flo- racion	Color flor	Habitus crecimiento	Posicion Vainas	Días Madurez	Días cosecha	Color tanfo brillo semilla	Califica- cion 45 y 65 días
56	1109 Méx	33	M	G	A	73	81	P-M-B	XX
58	1122 Méx	40	B	G	A	76	80	R-P-B	XXX
62	1153 Méx	41	M	G	A	86	98	P-M-B	XXX
63	1225 Méx	33	M	G	A	78	85	N-P-B	XX
66	1243 Méx	24	M	G	A	63	72	P-M-B	XX
74	1290 Méx	33	M	G	AM	74	85	N-M-B	XX
79	1326 Méx	41	B	G	A	80	91	P-M-B	XXX
90	1403 Méx	32	B	G	A	75	83	Ba-G-B	XXX
154	7-4-9	41	M	S G	B	71	76	N-M-N	XXX
167	1342 Méx 106	33	B	G	A	74	86	Ba-M-B	XXX
174	1410 Méx	32	B	G	A	70	81	Ba-M-B	XX

M - Morada C - Gula A - Alta P - Pinto N - Negro M - Mediano C - Grande
B - Blanca BG - Sembrada B - Baja R - Rojo Ba - Bayo P - Pequeno B - Brillante

WHITEFLIES ON BEANS IN THE WESTERN HEMISPHERE

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Five species of Aleyrodidae are known to occur on beans, Phaseolus vulgaris and Phaseolus lunatus, and on soybeans, Glycine max and Glycine hispida, in the Americas. They are Bemisia tabaci (Gennadius), Bemisia tuberculata Bondar, Tetraleurodes acaciae (Quaintance), Trialeurodes abutilonea (Haldeman) and Trialeurodes vaporariorum (Westwood). Of these, only Bemisia tabaci is known to be a vector of bean diseases. It transmits golden mosaic, yellow mosaic, mottled dwarf and perhaps other diseases of beans. Two other species are injurious, and all merit discussion because they live on beans.

Bemisia tabaci was injurious to tobacco in Greece when it was described in 1899. It is now a serious pest on 5 continents. It is a vector of diseases of beans, cassava, cotton, tobacco and many other plants. It also injures plants by feeding and by excreting honey-dew in which fungus grows and smothers the plant. It lives on numerous wild plants from which it reinfests cultivated crops. The species has been described under 11 specific names that are recognized as synonyms of tabaci. Names of races of tabaci, such as those proposed by Bird (1971), in my opinion, represent only biotypes.

Several workers have investigated the biology of tabaci and their findings are condensed on the following pages. Only one of the authors cited reared tabaci on beans.

The life cycle of tabaci varies considerably and is correlated with climatic and vegetative conditions. Normally eggs are deposited singly or in groups on the lower surface of the upper and newer leaves. In heavy infestations, eggs also may be found on the upper surface of leaves and on stems. The adult female penetrates the epidermis and mesophyll of the

leaf with her ovipositor and embeds the tip of the pedicel of the egg in the minute opening. Thus the eggs are firmly anchored in the leaf and remain viable as long as the leaves are alive. If the leaves die, however, the eggs also die unless the incubation period is nearly complete. In this case, survival is possible. Eggs are laid by mated females which produce both males and females and by virgin females whose eggs give rise to males only.

Costa and Bennett (1950) determined that in Brazil, in summer, on various plants, tabaci required 21 to 24 days to develop from the egg to the adult. In Puerto Rico, Bird (1957) found that 20 days were required for examples of tabaci caged on Jatropha gossypifolia to complete the life cycle.

Nene (1972), who worked with tabaci as a pest of pulse crops in India, indicated that the complete life cycle of tabaci on urd beans, mung beans and soy beans, required 13 to 24 days from March through November and 72 days December through January. Nene noted that adults declined on mature crops of the beans in October when adults started to move from them to crucifers, lentils, peas and other vegetables. The adults remained active but development was greatly reduced from November through March. In late February and March, adults moved back to urd, mung, soy beans, sweet potato, etc., and multiplied more rapidly as the weather became warmer.

Husain and Frehman (1933) studied the biology of tabaci, under the synonymous name Bemisia gossypiperda Misra and Lamba, on cotton in India. These workers gave the complete life cycle of tabaci as 14 to 107 days, the shortest periods being 14 to 21 days April to September, and the longest

periods usually 69 to 72 days from November to February. In a few cases the duration of the life cycle was 101 to 107 days.

Husain and Trehan stated that the average number of eggs laid per female was 25 one year and 43 another year and that the highest number laid by a female in captivity was 119 over a period of 18 days. The average number of eggs laid during a 24 hour period was 6 one year, 8 another year and the maximum number was 16. These workers observed that maximum oviposition occurred at temperatures above 26.5 degrees C and that oviposition stopped when the air temperature fell below 24 degrees.

Husain and Trehan gave the development periods of tubici as follows: oviposition period 2 to 18 days, incubation period 3 to 33 days, 3 to 5 days April to September, 5 to 17 days October and November, 7 to 16 days February and March, 33 days in December and January, the first 3 immature stages combined, 9 to 14 days April through September, 17 to 73 days during the remainder of the year and in a single instance the time was 81 days, pupal stage 2 to 8 days, adults in captivity 2 to 5 days in summer, up to 24 days in November. There were about 12 generations in the course of a year but since the brood overlapped, practically all stages were present at all times. They stated that temperature was the controlling factor in the development of tubici.

Husain and Trehan found that the varieties of cotton with which they worked were about equally acceptable to the whitefly but at different seasons of the year, that the plants were not equally infested during each season. They also found that the newest and the oldest plants were less heavily infested than others. This suggests a preference of the insect for a certain stage in the development of its host.

audibility of cell bag corresponds with
attach Raw PH, Low March 5

Avidov (1956) made an excellent, detailed study of the biology of tabaci on egg plant in Israel. His experiments were carried out in open air conditions in the laboratory for more than 2 years and for 25 continuous generations. Observations and surveys also were made in the field.

Avidov found that adults of tabaci were active soon after emergence and that in summer they mated within 1 to 8 hours, while in the spring and fall they mated 1 to 3 days after emergence. Copulation took place several times during the life of adults. The pre-oviposition period varied during different seasons of the year and was governed by temperature and humidity--on very hot, dry days, no eggs were laid and adults died. A relative humidity of 61 to 76 per cent did not affect the duration of the pre-oviposition period. The threshold for oviposition was about 14 degree C, that for development of the eggs was 12.5 degrees, while that of the immature stages was 12 degrees C.

During the summer months, hatching of the larvae occurred within a few minutes after egg deposition, but in the winter, hatching sometimes took several hours. The larvae crawled for a very short distance and within a few hours in summer and a few days in cooler weather, settled permanently, inserted their stylets and began to feed. Firmly anchored to the leaf, they were not greatly affected by extreme weather conditions or by relative humidity as long as the leaves were turgid.

The duration of the development stages of tobacco for each month of the year is given in the following table by Avidov

TABLE NO. (XV) OF THE DEVELOPMENT STAGES IN THE TOBACCO WHITE FLY
IN VARIOUS MONTHS OF THE YEAR

Month	Mean temp (C)	Developmental stages			Total development
		Pre oviposition period	Egg stage	Larval stage	
January	1.2	—	11—19	20—32	10—51
February	13.8	0—22	1—19	—	10—43
March	12.5	—	—	21—30	11
April	12.1	5—14	9	1—11	15—21
May	12	3—6	6—7	9—15	15—23
June	12	1—7	5—7	9—11	12—18
July	20.0	2—6	4—8	7—11	11—18
August	26.5	—3	4—5	7—11	13—17
September	25.1	2—4	6	7—11	15—20
October	12	1—8	—	9—10	2—23
November	20.6	6—22	8—22	15—16	21—4
December	16.5	—	—	17—60	22—65
Temp. threshold		14° C	15° C	16° C	

As shown, the life cycle was shortest, 13 to 17 days, in August, when the mean temperature was highest, 26.5 degrees C. At that time the pre-oviposition period was 2 to 3 days, the egg stage 4 to 8 days and the larval stages 7-11 days. The longest total development period was 55 to 75 days in December when the mean temperature was 16.5 degrees C. The length of the preoviposition and egg stages were not indicated for December, but the larval stages lasted 42 to 60 days in that month.

Avidov gave the seasonal variation in the longevity of adults in the following table

SEASONAL VARIATION IN ADULT LONGEVITY
(DAYS)

Month of emergence	Mean temp (C)	Males			Females		
		adults No. of	Longevity av	max	No. of adults	Longevity av	max
February	13.5	18	10.1	15	22	43.5	67
April	14.1	26	11.2	29	20	18.6	31
May	21.7	24	14.5	21	28	22.5	39
June	1	22	6.4	10	36	14.5	16
July	6.0	0	0.1	17	20	20.0	58
August	6.5	16	10.5	21	18	21.0	36
September	25.4	16	3.0	3	15	17.0	3
October	21.2	38	6.8	15	22	31.0	41
November	0.6	18	1.5	37	35	25.0	15
December	16.5	8	34.0	54	12	55.3	9

The minimum longevity of females was 23 days and of males was 3 days in September when the mean temperature was 25.4 degrees C. The maximum longevity of females was 78 days while males lived only 54 days in December when the mean temperature was 16.5 degrees C.

Avidov found that adults that emerged in December overwintered and started to oviposit in the spring when temperature was above 14.5 degrees C. When the relative humidity averaged below 60 percent, females stopped ovipositing and many died prematurely. Development ceased below 12 degrees C.

Avidov stated that the number of generations of tabaci varied between 11 and 15 per year in different parts of Israel. In the sequence of generations studied by Avidov in the laboratory, the shortest life span was 13 days in July and the longest was 84 days from November to February.

SEQUENCE OF GENERATIONS IN TOBACCO WHITE FLY

Generation	Occurrence (1st year)	Life span (days)	Occurrence (2nd year)	Life span (days)
1st	Nov 3—Nov 11	—	Oct 29—Nov 11	23
2nd	Dec 1—Dec 24	61	Nov 23—Feb 7	84
3rd	Feb 19—Apr 8	4	Feb 7—Apr 4	61
4th	Apr 6—May 7	31	Apr 20—May 18	28
5th	May 7—May 27	20	May 21—June 6	22
6th	May 29—June 16	18	June 15—July 3	18
7th	June 24—July 15	21	July 5—July 24	19
8th	July 18—July 31	13	July 24—Aug 8	18
9th	Aug 3—Aug 27	19	Aug 11—Aug 30	19
10th	Aug 32—Sept 4	8	Sept 6—Sept 25	22
11th	Sept 16—Sept 27	17	Sept 28—Oct 10	27
12th	Oct 7—Oct 30	23	—	—

Although Avidov did not work with tabaci on beans, he reported the insect from Phaseolus vulgaris, Phaseolus lunatus, Pisum sativum, Cajanus indicus, Vigna sesquipedalis and Vigna sinensis.

The observations of the several workers cited show that the duration of the life cycle of tabaci is fairly similar in the countries where it has been worked out.

A species that I have identified as Lemisia tuberculata Bondar has been found on soy beans in Peru. This species as I understand it, is as variable as is tabaci. It is also very close to tabaci, but I believe the two are distinct species.

Lemisia tuberculata was reported to be a possible vector of tobacco leaf curl in Venezuela by Wolf, Whitcomb and Mooney (1949). Its biology apparently has not been investigated, but I suspect it would be similar

to that of tuberculatus. I have not seen heavy infestations of tuberculatus

Tetraneura, viridis (Quaintance) is a common species on trees and shrubs of the Leguminosae in warmer areas of the United States (Arizona, California, Florida, Texas), Mexico, Central America, and the West Indies. And I have seen a few examples from Surinam and Venezuela. I have seen only 2 collections of this species from beans, 1 from Mexico and 1 from Guatemala.

The species has not been recorded as a vector of any disease. The life cycle of acaciae has not been studied.

Trialeurodes abutilonea (Haldeman) is a common species on many plants in the United States, and it also occurs in Mexico, Central America and the West Indies. It is found on green beans and soy beans in the United States but I have not seen it on beans from other areas. Carman and Jewett (1922) stated that indoors it had a preference for leguminous plants.

Laird and Dickson (1959) were not able to transmit the leaf crumple of cotton with this whitefly, and Dysart and Chamberlain (1960) failed to transmit tobacco ringspot virus from infected to healthy soy bean plants with two species. However, Hildebrand (1959) reported that abutilonea was a vector of sweet potato feathery mottle in Maryland, USA, though later (1960) he stated that the whitefly transmitted only one component, yellow dwarf, of feathery mottle.

The biology of abutilonea was investigated by Dysart (1961) on Abutilon in an outdoor insectory in Illinois, USA. Dysart stated that the egg stage lasted 5 to 8 days, and the immature stages 6 to 16 days. The life span of adults and the total life span were not indicated.

Trialeurodes vaporariorum (Westwood), known commonly as the green-

house whitefly is known throughout the world as a pest of numerous plants. It has long been harmful in greenhouses in Europe and North America, and in recent years has become a serious pest out-of-doors in warm and in temperate, climates during summer months.

Trialeurodes vaporariorum apparently has not proved to be a vector of any virus disease. ^(Hew) Dysart and Chamberlain (1960) were unable to transmit tobacco ringspot virus from infected to healthy soybean plants with this aleurodid.

The habits and biology of vaporariorum have been investigated by several persons including Morrill (1903, 1905), Hargreaves (1915), Garman and Jewett (1922) and Weber (1931). Their studies were conducted in greenhouses on various plants.

Morrill (1903, 1905) stated that the incubation period of the egg was 10 to 12 days, the first larval stage 5 to 7 days, the second and third stages each 4 to 6 days, the fourth stage 13 to 16 days, and the adults 23 and 36 days in the cases that were observed. Morrill indicated that the entire life span of vaporariorum usually was nearly 5 weeks.

Garman and Jewett (1922) found that the egg stage of vaporariorum lasted 6 to 13 days, the first and second larval stages 2 to 8 days, the third stage 2 to 6 days, and the fourth stage 6 to 13 days. These authors did not give the life span of adults. The longest life cycle they observed was 46 days, but the average cycle was 21 to 41 days.

Hargreaves (1915) gave longer periods for each stage, 11 to 14 days for the egg, 10 to 12 days for the first stage larva, 14 to 22 days for the second stage, 5 to 36 days for the third stage and 21 to 59 days for the fourth stage. Weber (1931) pointed out that the 14 to 22 days given

by Hargreaves as the duration of the second stage were valid for sub-optimal temperatures. In Weber's own experiments, at optimum temperatures of 25 to 30 degrees C, the second larval instar lasted 1 to 2 days, and at 19 degrees C it lasted 3 days. Weber found that the third larval stage took longer, 2 days at 29 degrees C, 3 to 4 days at 20 to 23 degrees, 6 days at 16 to 19 degrees. At 8 degrees C, development ceased in the second and third stage larvae and in eggs. Weber indicated that temperature and relative humidity were important factors in the life and mortality of the various stages of the greenhouse whitefly.

Pupae of the 5 species that I have discussed can be separated into 3 recognizable groups on the basis of their appearance on their host plants. Larvae of the 3 groups also are sometimes distinguishable, but most adults are not. It should be remembered that the characteristics that distinguish the 3 groups may not separate them from other species that may eventually be found on beans.

Tetraleurodes seneciae represents one group. The pupae of this species are black, convex dorsally with a depressed line around the submargin. The body is encircled by a white, slightly flocculent, waxy secretion that extends outward from the margin. Perfect specimens have a little white wax on the dorsum, but this frequently is destroyed. The color, the convexity of the body and the nature of the waxy secretion of the insect are entirely distinct from the other species treated here. The larvae also are black and convex dorsally.

Slide of Tetraleurodes seneciae

Bemisia tabaci and Bemisia tuberculata represent another group.

These species are colorless or very pale yellowish or greenish, and their

waxy secretion is colorless, very thin and usually is apparent only under magnification. The body outline of tubici and tuberculata varies, but it tends to taper toward the posterior end and to be slightly narrower there than at the anterior end. The dorsal surface is flat or very slightly convex. The larvae of these 2 species are colorless or slightly yellowish or greenish.

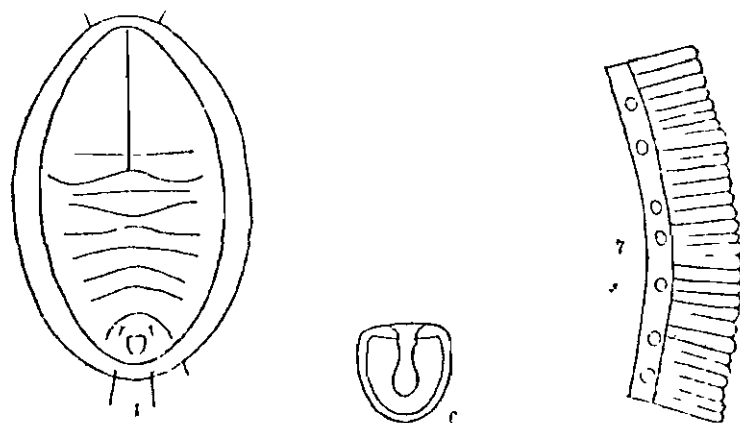
Slides of Bemisia tubici and tuberculata

Trialeurodes abutilonea and Trialeurodes vaporariorum represent the third group. These species are actually or nearly flat dorsally and bear conspicuous waxy secretions. Glassy, colorless rods of wax arise from the body, there is a thin layer of similar wax on the dorsum, and in mature pupae a vertical piloside of thin, translucent wax extends from the body margin to the surface of the leaf. These species are subelliptical in outline. The body is colorless or pale yellowish, or abutilonea may be brown in the median area of the dorsum. Larvae of these species also are subelliptical in outline and can sometime be separated from Bemisia by their shape.

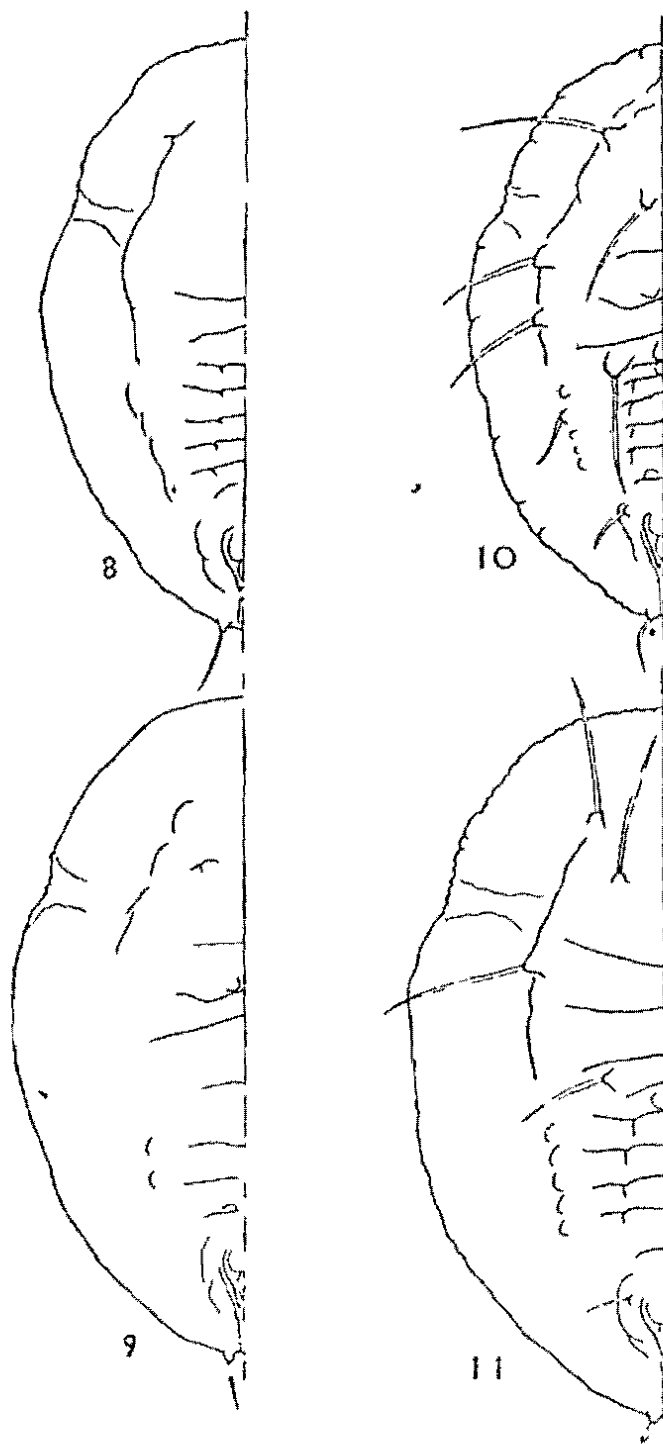
Slides of Trialeurodes abutilonea and vaporariorum

Accurate determinations of the species discussed here can be made only from well prepared, slide mounted specimens. Examples of Tetraleurodes acaciae must be bleached, while the other species should be stained for best results. The species of Bemisia and Trialeurodes exhibit extensive variation in some structures and in such cases, experience is an asset in determining the insects. Variation in structure frequently can be correlated with the physical properties of the leaf on which the

insects developed. Such variation is evident in the illustrations of Bemisia tabaci, Trialeurodes abutilonea and Trialeurodes vaporariorum. The illustration of tetraneura acaciae serves only to show differences between this and the other species discussed here.



Tetrileurodes aciculae, after Quintance



FIGS 8-11—Pupal cases from (8) cotton (9) *Delich* s. (10) *Centrosema* (11) tobacco
Pemisia tabaci, after Mound

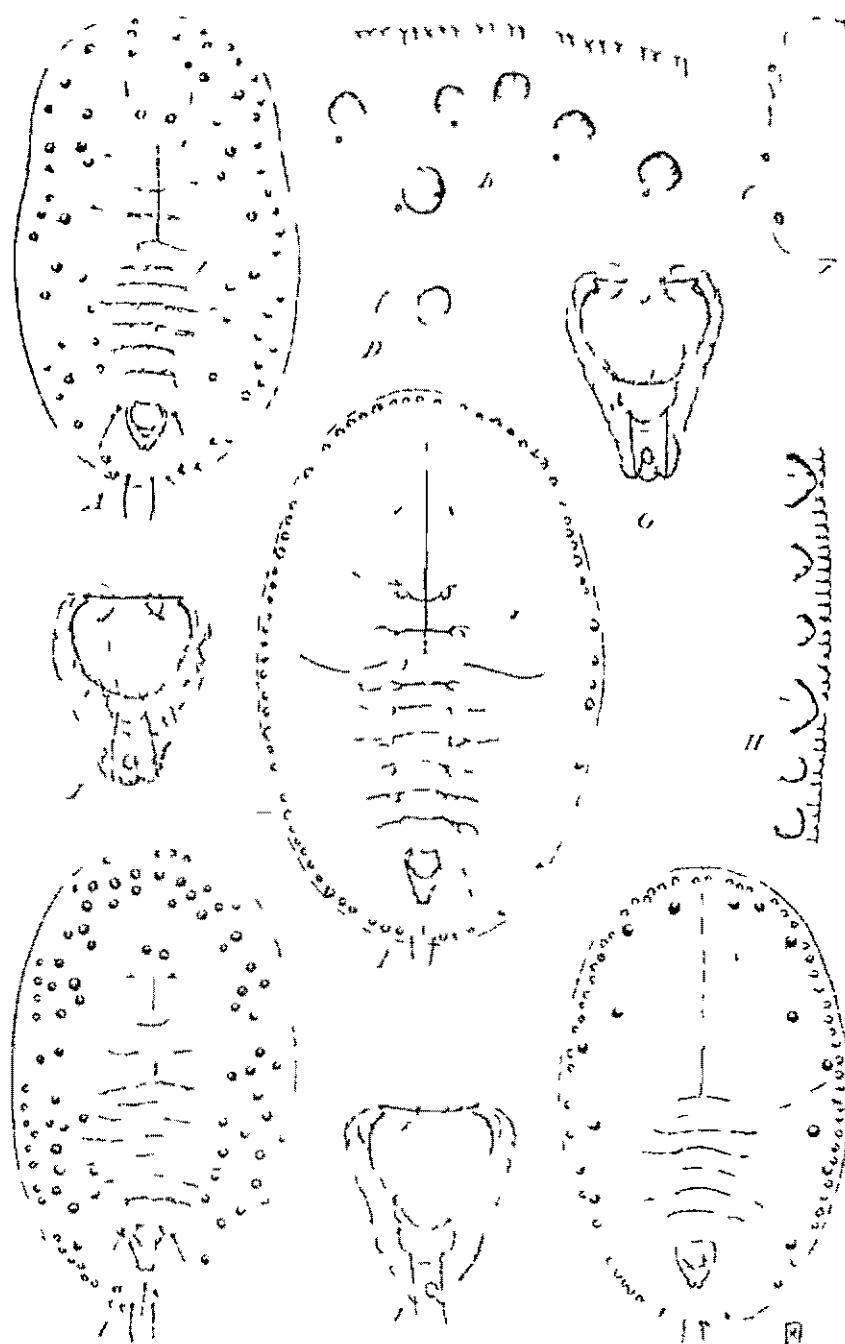


FIGURE 31. *Trioxys* *glabellus* (A-G) and *Trioxys* *glabellus* (H-I) from the United States. *Trioxys* *glabellus* (J-K) from the United States. *Trioxys* *glabellus* (L-M) from the United States. *Trioxys* *glabellus* (N-O) from the United States. *Trioxys* *glabellus* (P-Q) from the United States.

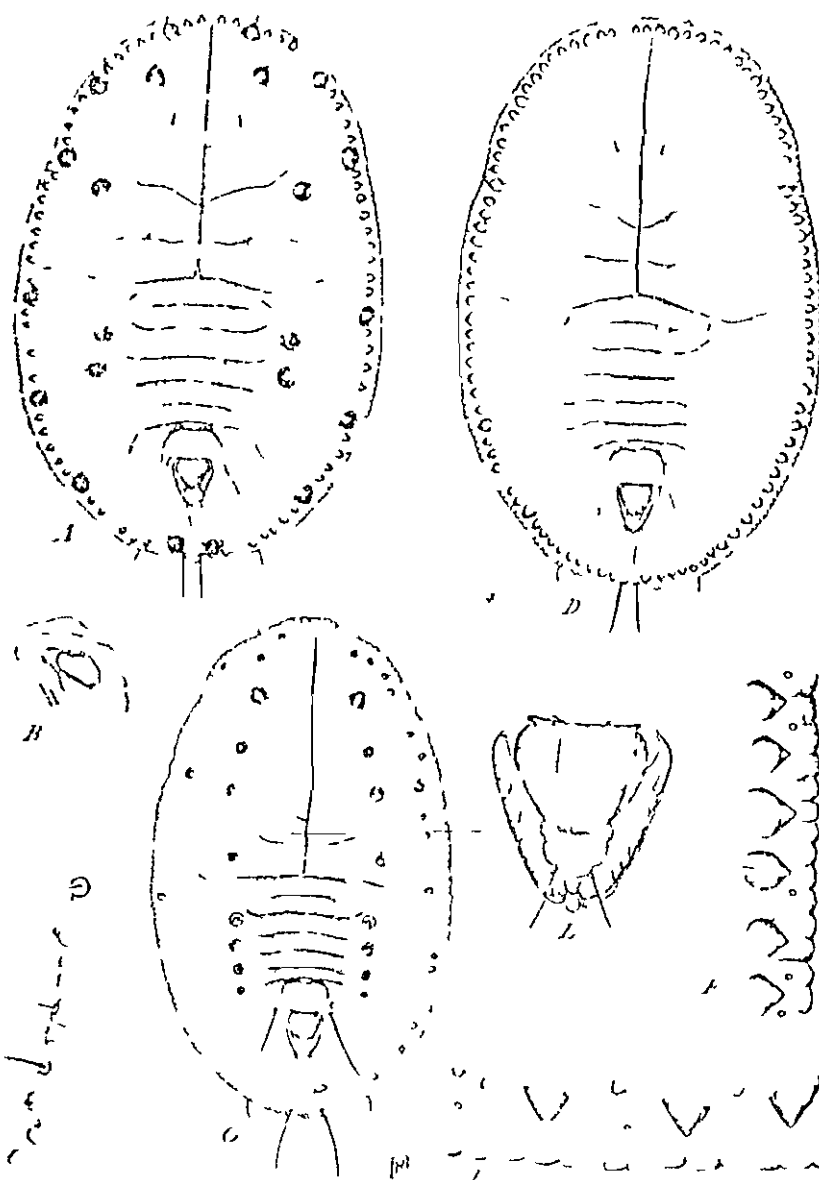


FIGURE 18. *Myrmica* sp. (1) On the ground from moderately
 hairy leaf. (2) One thereof, part of a marginal cream
 tubercle. (3) Outline of specimen from a leaf. (4) Ventral view of
 specimen of marginal tubercle. (5) Lateral view of very
 hairy leaf. (6) Section of marginal tubercle of specimen from a
 tubercle. (7) Section of marginal tubercle of specimen from a
 tubercle.

The following key, combined with the illustrations, should be of help in separating the 5 species. Please refer to the illustrations

- 1 Pupae dark brown or black, margin with strong teeth, well defined ridges extending from marginal teeth to submarginal ridge, with a submarginal ridge bearing a single row of disklike pores, with a furrow just mesad of the submarginal ridge, operculum filling the vasiform orifice and concealing the small spatulate lingula

tetraleurodes acutic (Quintance)

- | | |
|--|---|
| Characters not as above | 2 |
| 2 Without submarginal or other papillae, lingula not lobed, body margin not uniformly crenulate, body outline sub-ovoid | 3 |
| With submarginal and with or without dorsal papillae, lingula lobed, body margin uniformly crenulate, body outline subelliptical | 4 |

3. Widest part of vasiform orifice as wide or slightly wider than length of the caudal furrow, with a median tooth at distal end of vasiform orifice and usually with 1 to 3 teeth at anterior end of caudal furrow, operculum broadly curved around posterior margin, pupae 0.60 to 0.85 mm long and 0.45 to 0.60 mm wide

Bemisia tabaci (Gennadius)

Widest part of vasiform orifice about one-third less than length of caudal furrow, without a median tooth at distal end of vasiform orifice or at anterior end of caudal furrow, but with 1 or more transverse lines in these areas, operculum narrowly curved around posterior margin, pupae 0.75 to 1 mm long and 0.60 to 0.75 mm wide

Bemisia tuberculata Bonnier

4. Minute pores mesad of submarginal papillae, papillae broadly curved at apex, marginal crenulations narrow, about 30 in 100 μ , pupae usually 0.60 to 0.90 mm long and 0.45 to 0.60 mm wide Trileurodes abutilonea (Haldeman)

Minute pores distad of submarginal papillae, papillae narrower and sharper at apex, marginal crenulations wider, about 12 in 100 μ , pupae usually 0.75 to 1.10 mm long and 0.50 to 0.75 mm wide Trileurodes vaporariorum (Westwood)

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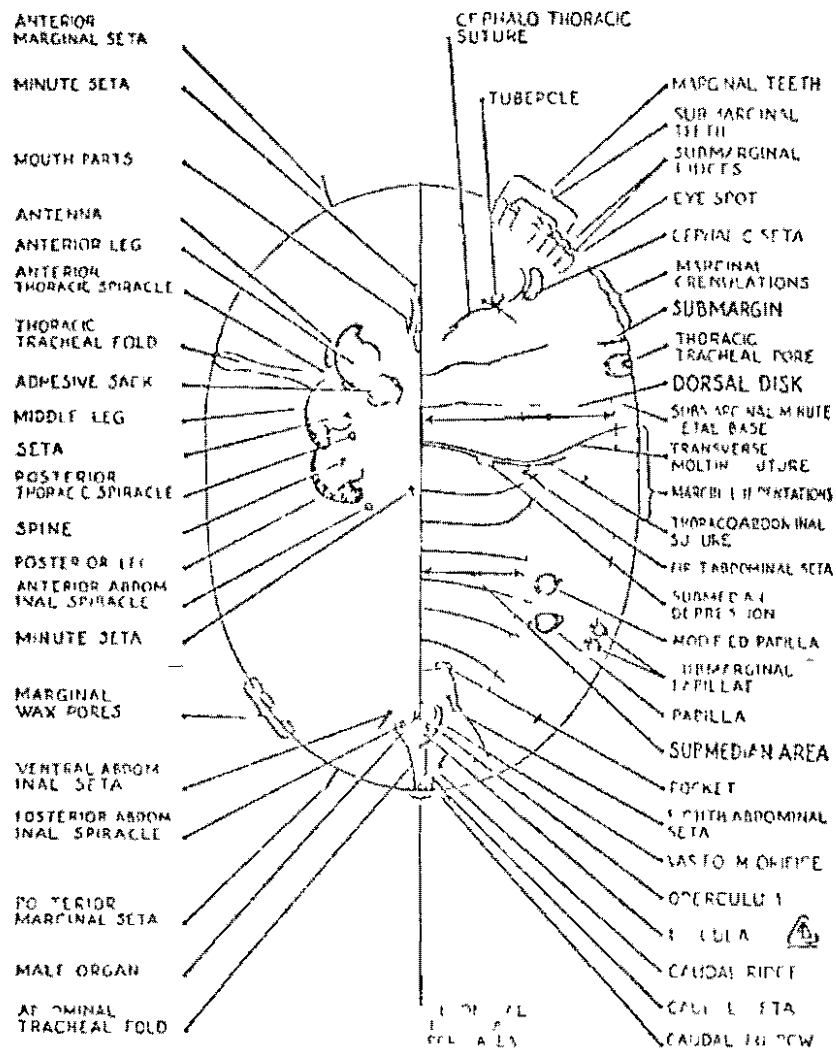


Fig. 1. Fly (Diptera) showing the anatomy of the fly.

Breeding for resistance to Empoasca kraemer.

A v Schoonhoven
CIAT, November 1975

1.- Introduction

Resistance to insects in plants is defined by Painter (1951) as the heritable characteristic of a variety of plant species to produce a larger crop of a better quality than other varieties at the same level of insect population.

In this definition resistance is limited to differences within a plant species. Difference in insect attack among species fall in the category of host-plant selection. The definition also says that the characteristic is inherited from parents to offspring. Apparent resistance may also be due to for instance a fertilizer response or escape from attack and are examples of noninherited resistance.

Resistant plant varieties have many advantages over those which are not resistant. Insects are controlled without intervention in other species, there is no pollution and once the resistant variety is developed, it can be used without extra costs which is of special importance to low income producers.

Some of the disadvantages of resistance are the time and cost involved to develop a resistant variety, which is however much cheaper than the cost of the development of an insecticide. Very few examples are known where resistance to an insect has broken down. This is in contrast to pathology, where new races often are formed as rapidly as new resistant varieties are produced. In parthenogenetic reproducing insects some examples are known of lost resistance.

2 - Resistance Mechanisms

Resistance, as observed in the field was divided by Painter (1951) into three mechanisms based on insect-host-plant interaction. Mostly a combination of these 3 mechanisms is present in resistance. These are

- a.- Non-preference a variety is less preferred than another variety for oviposition, feeding or shelter.
- b - Antibiosis a variety has an adverse effect on the biology of the insect. This is probably the least desirable form of resistance as it places a selection pressure on the insects.
- c - tolerance This is the ability of a plant variety to repair, recover or withstand insect attack. This is the most favorable and natural form of resistance in my opinion, as it does not create selective forces to break resistance. Plants

and insects in their individual struggle for survival have found a status in which no evolutionary forces to break this status are present. It also permits continuous insect populations as hosts for parasites and predators. It requires however training of the crop grower to convince him that no economic losses will result, within certain limits (the economic threshold population), despite the presence of insects.

3.- Literature on resistance in beans to *E. kraemer*

In the literature, eg Gutierrez et.al. (1975), *E. kraemer* is reported as the principal bean pest in Latin America. It does not transmit virus diseases but it is suspected that it induces growth regulating substances in the plant tissue. The wide distribution of the leafhopper and its high population numbers, especially in dry season conditions, contribute to its importance.

The literature on *E. kraemer* is limited, while most research has been done on *E. fabae*. The two species were separated in 1957 by Ross and Moore and *E. fabae* seems to be limited to the USA while *E. kraemer* is reported from Florida, Latin America and the Caribbean. *E. kraemer* does not transmit virus

diseases in beans. The only leafhopper known to transmit virus diseases in beans in America is the beet leafhopper Circulifer (= Eutettix) tenellus, which transmits curly top. Resistant varieties to the beet leafhopper like Idaho Refugee (reported resistant to E. fabae) Burpee Stringless Greenpod and Landreth Stringless Greenpod do also have reduced virus incidence. (Hallock, 1946).

Chalfant (1965) tested 28 varieties for leafhopper (E. fabae) resistance. He found a highly significant positive correlation ($r = 0.64$) between insect counts and damage scores. The number of nymphs per leaf ranged from 0.15 (on Topcrop) to 2.21 (on White Half Runner), while the damage on a 0-10 scale ranged from 0.4 (C 14) to 3.5 for Mountaineer.

McFarlane and Keenan (1943) tested 27 varieties of Ph. vulgaris for E. fabae resistance. They found Henderson Bush Lima most resistant while Idaho Refugee ranked 3rd. The resistance ratings of the same varieties over the 4 replicates was very uniform, indicating homogeneity of infestation.

Most research on resistance in beans to E. fabae has been published by Wolfenbarger and Sleesman in 1961. They also found a highly significant positive correlation between hopperburn rating and nymphal counts ($r = 0.90$), however they also found

tolerance to nymphal attack. This was expressed in FM-52 which had a hopperburn rating of 0, while the nymphal population per leaf reached 15.0. They value resistance scores based on nymphal population higher than hopperburn ratings as this also included preference for oviposition. Low nymphal counts were obtained from PI 151-014 and PI 173 - 024 with 0.3 and 0.4 nymphs per leaf, respectively, while Dutch Brown with 19.7 nymphs per leaf was the highest recorded. They obtained a correlation of $r = -0.19$ (n.s.) for number of epidermal hairs and nymphal population. Therefore hair density was not related to resistance but hair type still may be. In other Phaseolus species they also found great differences in resistance ranking among the varieties. High levels of resistance were found among Ph. aureus and Ph. lunatus and Ph. radiatus sources. They found in interspecific crosses between resistant and susceptible materials indications that resistance was recessive. Plant characteristics that were studied for association with resistance showed, that pod color, leaf area, and growth habit were uncorrelated with nymphal counts, however taller plants had significantly fewer nymphs, and also intermediate maturing varieties and those possessing mosaic resistance were more resistant. Pink seeded varieties were more resistant than those with a mottled seed color.

Medina and Guerra (pers. comm.) found Empoasca resistance, in Negro 66 and Canario 101, which were also resistant to the Mexican bean beetle and the bean pod weevil. Avalos (pers. comm.) also found large differences in Empoasca resistance among bean lines, with some lines giving equal yields in protected and non-protected plots

4 - CIAF's Empoasca kraemer resistance program

CIAF's entomology program has as main objective to reduce the economic importance of pests by increasing the resistance to these pests in new bean varieties. It is hoped that in this way the need for insecticides will be reduced and that in absence of chemical control measures a reasonable yield can still be obtained

Development of resistance to Empoasca kraemer, the principle bean pest of Latin America, is the first objective in our program

To achieve above objectives for E. kraemer varieties were screened for resistance to E. kraemer. First commercial varieties were tested, and as the levels of resistance found were not high enough, resistance sources mentioned in the literature were tested, followed by the screening of the CIAF's germplasm bank (about 8000 have been screened, which is the available

part). Until now we have not encountered levels of resistance high enough to ensure good yields under high leafhopper populations. Some attention is paid to levels of resistance in other crossable species with Ph. vulgaris, like Ph. coccineus. However the main emphasis is placed on a large hybridization program within Ph vulgaris to raise the level of resistance, or to combine different resistance mechanisms to raise resistance in this way

After screening the available material in the germplasm bank of CIAT, 395 entries were selected for advanced testing. These entries will be planted in replicated plots to score — damage, in the form of hopperburn and to make nymphal counts and instar distribution of the nymphs, which we hope will indicate antibiosis type of resistance. Until now, field tolerance was the main type of resistance selected for. Some materials, reported resistant, were not classified among the most resistant entries.

Mechanisms of resistance

Lines selected for resistance in the field did show average leafhopper populations. In detailed laboratory tests with few varieties we found a significant ovipositional non-preference in ICA-Tui, a black seeded variety being least preferred. But

placing the varieties individually, in cages without choice, no differences were found in oviposition rate, indicating a low level of non-preference. The non-preference was as well for oviposition as for feeding when tested with males only. Antibiosis in these selections and in 54 additional ones tested, was not found.

The level of resistance found so far is sufficient in the wet season, when leafhopper populations are generally low. Selection 73 Vul 3624 yielded equal with and without insecticidal protection, but in the dry season under high population pressure the yield increased 4.2 fold following protection. This clearly indicated that resistance is present, especially when data are compared with susceptible lines, which gave a 3 and 36 fold yield increases in the wet and dry season, respectively. The 36 fold yield increase was obtained with the most popular Colombian variety, Diacol-Calima.

5.- Future research

Fourteen of our best selections entered a diallel crossing program to measure which crosses give best increase in resistance (combining ability). Individual plants, selected in the F_2 will be crossed and tested again. With this recurrent selec-

tion procedure we hope to raise the level of resistance sufficiently. Also crosses are being made between the 14 selections and a susceptible variety to test inheritance of resistance and detect different genes for resistance.

Studies are underway to refine screening procedures both to better screen individual plants in segregating populations and to make the evaluation scale sufficiently sensitive to detect small differences in levels of resistance. When the level of Empoasca resistance can be raised sufficiently by hybridization and selection, more emphasis will be placed on similar programs for leaf feeding beetles (Diabrotica, Cerotoma, etc) and mites.

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Phaseolus crosses, Phaseolus spp , the cowpea .
and the bonavist bean. J Econ. Entomol. 54:
1077-1079.

waxy secretion is colorless, very thin and usually is apparent only under magnification. The body outline of tubaci and tuberculata varies, but it tends to taper toward the posterior end and to be slightly narrower there than at the anterior end. The dorsal surface is flat or very slightly convex. The larvae of these 2 species are colorless or slightly yellowish or greenish.

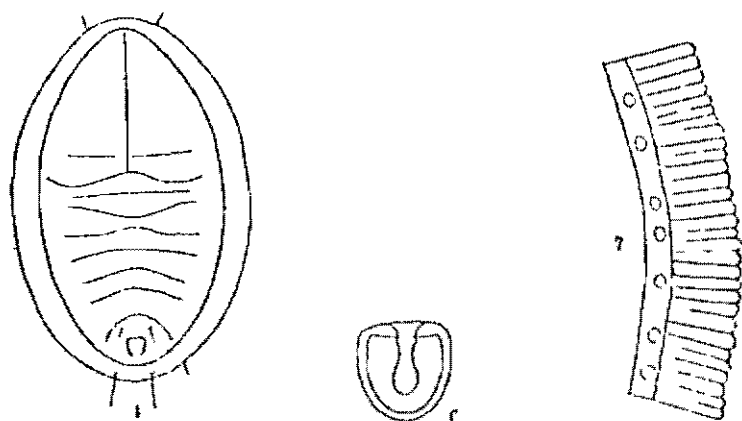
Slides of Bemisia tubaci and tuberculata

Trialeurodes abutilonae and Trialeurodes vaporariorum represent the third group. These species are actually or nearly flat dorsally and bear conspicuous waxy secretions. Glassy, colorless rods of wax arise from the body, there is a thin layer of similar wax on the dorsum, and in mature pupae a vertical palisade of thin, translucent wax extends from the body margin to the surface of the leaf. These species are subelliptical in outline. The body is colorless or pale yellowish, or abutilonae may be brown in the median area of the dorsum. Larvae of these species also are subelliptical in outline and can sometimes be separated from Bemisia by their shape.

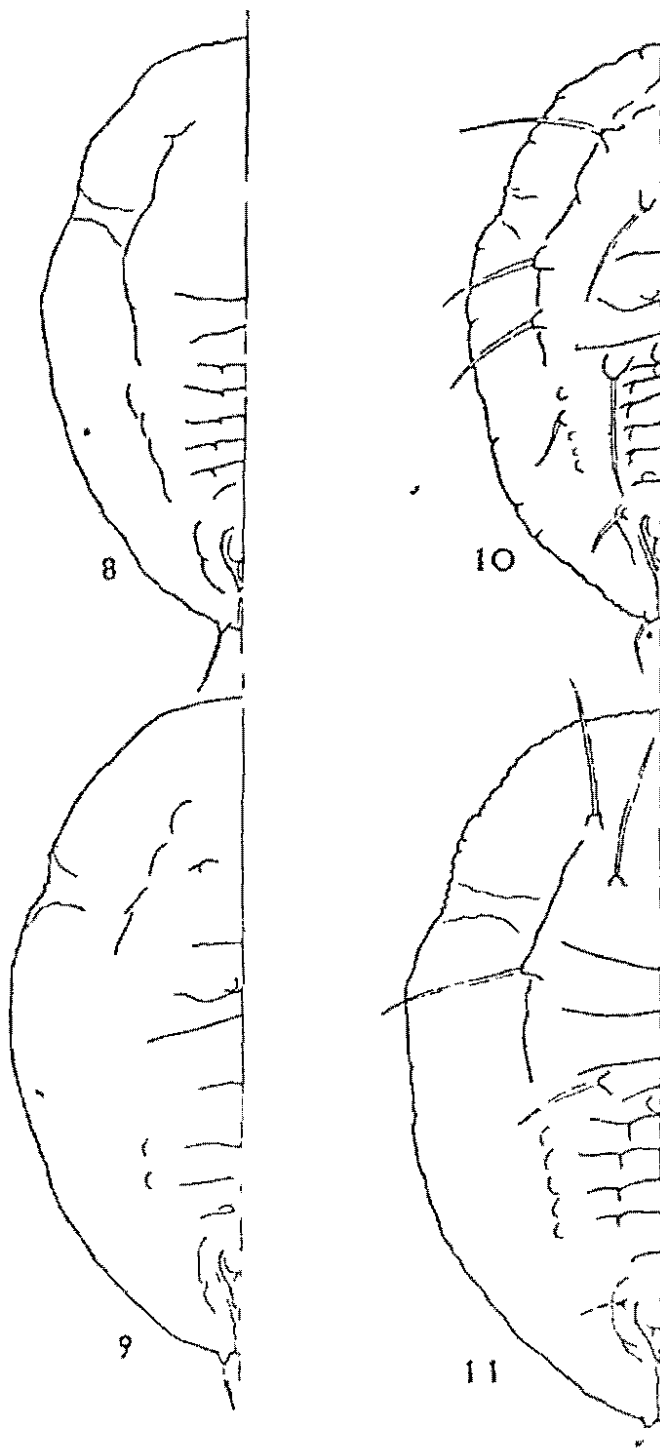
Slides of Trialeurodes abutilonae and vaporariorum

Accurate determinations of the species discussed here can be made only from well prepared, slide mounted specimens. Examples of Trialeurodes acaciae must be bleached, while the other species should be stained for best results. The species of Bemisia and Trialeurodes exhibit extensive variation in some structures and in such cases, experience is an asset in determining the insects. Variation in structures frequently can be correlated with the physical properties of the leaf on which the

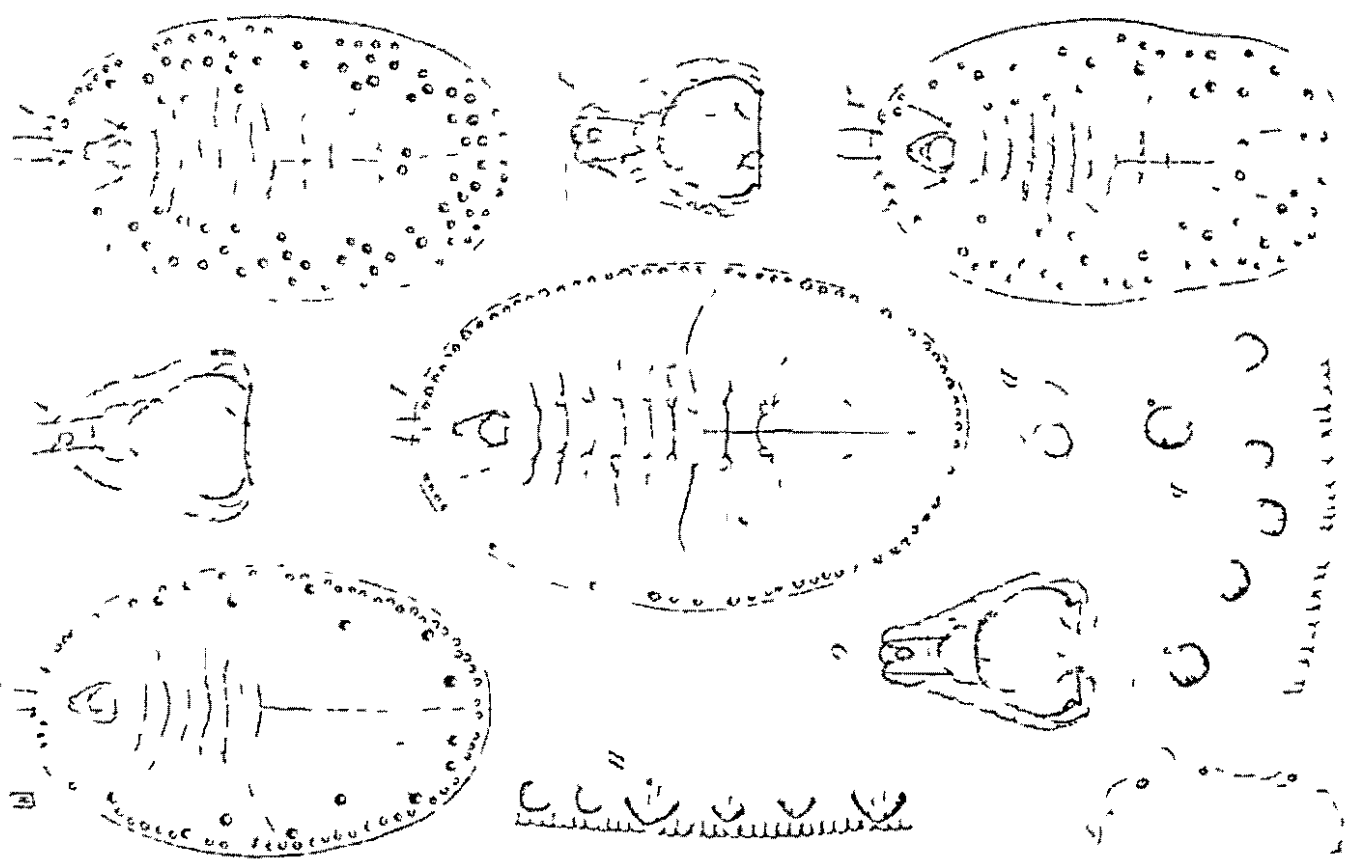
insects developed. Such variation is evident in the illustrations of Bemisia tabaci, Trialeurodes abutilonae and Trialeurodes vaporariorum. The illustration of tetraleurodes acaciae serves only to show differences between this and the other species discussed here.



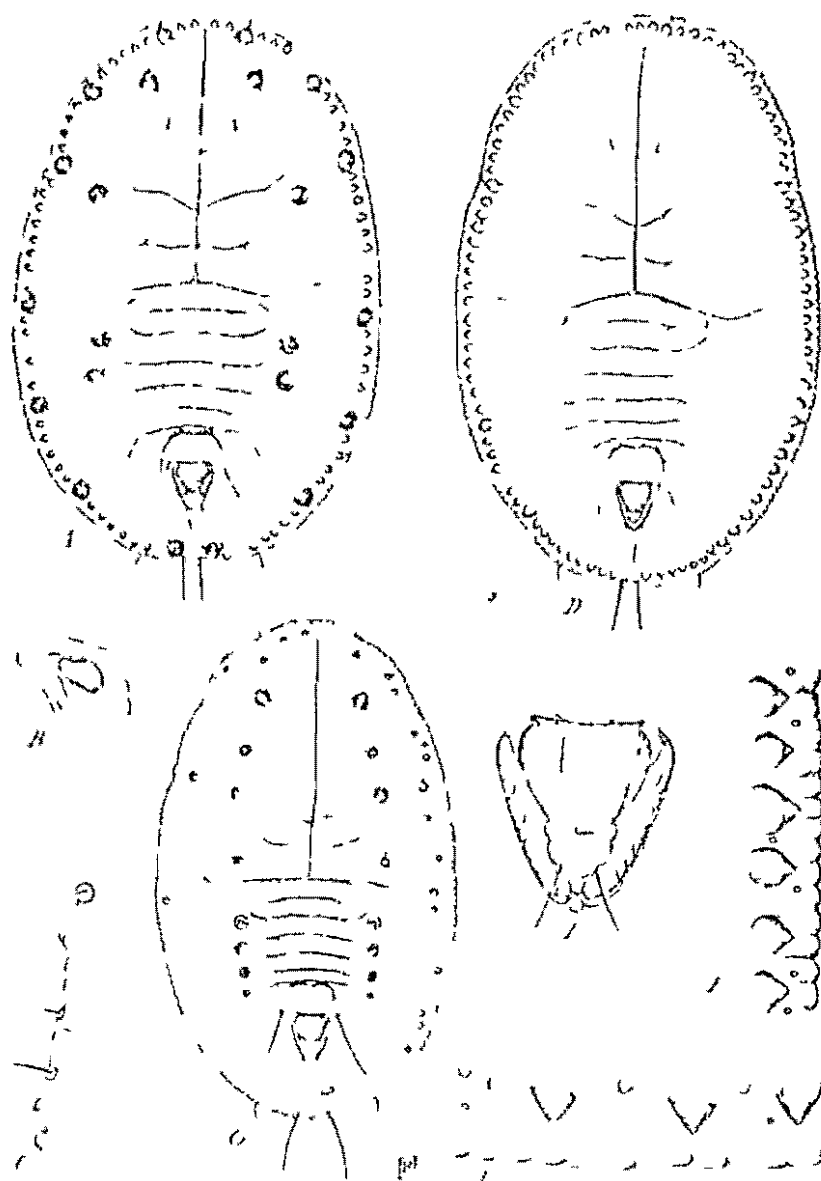
Tetrileurodes aciculae, after Quintance



FIGS 8-11.—1 up of cases from (8) cotton (9) *Centrosema* (10) *Centrosema* (11) tobacco
Bemisia tabaci, after Mound



The first of the three dorsal views of the beetle is a *Hydrophilus* sp. The second is a *Hydrophilus* sp. The third is a *Hydrophilus* sp. The head views are of *Hydrophilus* sp. The antenna is of *Hydrophilus* sp. The segmented structure at the bottom is of *Hydrophilus* sp.



From 1880 to 1900, the population of the United States grew from 31 million to 76 million. The growth was rapid, and the population of the United States was one of the most rapidly growing in the world. The growth was due to a number of factors, including immigration, a high birth rate, and a low death rate. The population of the United States was one of the most rapidly growing in the world.

The following key, combined with the illustrations, should be of help in separating the 5 species. Please refer to the illustrations

- 1 Pupae dark brown or black, margin with strong teeth, well defined ridges extending from marginal teeth to submarginal ridge, with a submarginal ridge bearing a single row of disklike pores, with a furrow just mesad of the submarginal ridge, operculum filling the vasiform orifice and concealing the small spatulate lingula

tetraleuroder acaciae (Quintance)

Characters not as above

2

- 2 Without submarginal or other papillae, lingula not lobed, body margin not uniformly crenulate, body outline sub-ovoid

With submarginal and with or without dorsal papillae, lingula lobed, body margin uniformly crenulate, body outline subelliptical

4

3. Widest part of visiform orifice as wide or slightly wider than length of the caudal furrow, with 1 median tooth at distal end of visiform orifice and usually with 1 to 3 teeth at anterior end of caudal furrow, operculum broadly curved around posterior margin, pupae 0.60 to 0.85 mm long and 0.45 to 0.60 mm wide

Bemisia tabaci (Gennadius)

Widest part of visiform orifice about one-third less than length of caudal furrow, without a median tooth at distal end of visiform orifice or at anterior end of caudal furrow, but with 1 or more transverse lines in these areas, operculum narrowly curved around posterior margin, pupae 0.75 to 1 mm long and 0.60 to 0.75 mm wide

Bemisia tuberculata Bondar

4. Minute pores mesad of submarginal papillae, papillae broadly curved at apex, marginal crenulations narrow, about 30 in 100 μ , pupae usually 0.60 to 0.90 mm long and 0.45 to 0.60 mm wide

Trileurodes abutilonae (Haldeman)

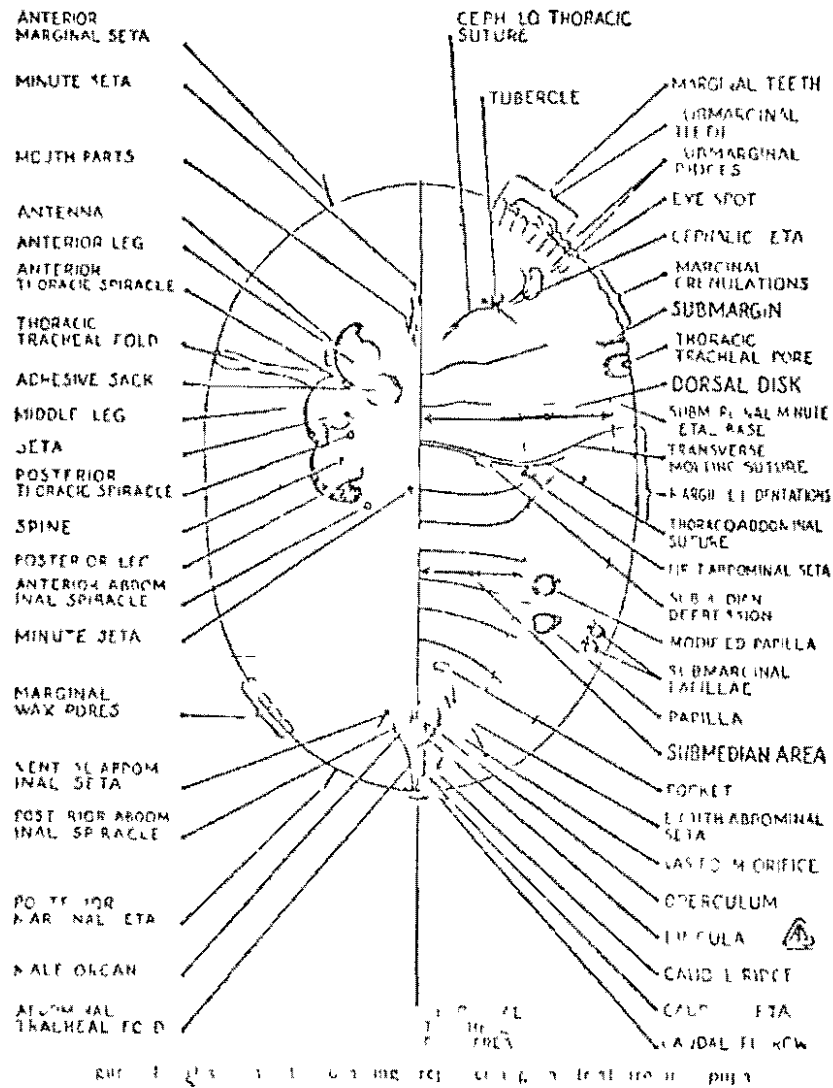
Minute pores distad of submarginal papillae, papillae narrower and sharper at apex, marginal crenulations wider, about 12 in 100 μ , pupae usually 0.75 to 1.10 mm long and 0.50 to 0.75 mm wide

Trileurodes vaporariorum (Westwood)

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Breeding for resistance to Empoasca kraemerl.

A v. Schoonhoven
CIAT, November 1975.

1.- Introduction

Resistance to insects in plants is defined by Painter (1951) as the heritable characteristic of a variety of plant species to produce a larger crop of a better quality than other varieties at the same level of insect population.

In this definition resistance is limited to differences within a plant species. Difference in insect attack among species fall in the category of host-plant selection. The definition also says that the characteristic is inherited from parents to offspring. Apparent resistance may also be due to for instance a fertilizer response or escape from attack and are examples of noninherited resistance.

Resistant plant varieties have many advantages over those which are not resistant. Insects are controlled without intervention in other species, there is no pollution and once the resistant variety is developed, it can be used without extra costs which is of special importance to low income producers.

Some of the disadvantages of resistance are the time and cost involved to develop a resistant variety, which is however much cheaper than the cost of the development of an insecticide. Very few examples are known where resistance to an insect has broken down. This is in contrast to pathology, where new races often are formed as rapidly as new resistant varieties are produced. In parthenogenetic reproducing insects some examples are known of lost resistance.

2 - Resistance Mechanisms

Resistance, as observed in the field was divided by Painter (1951) into three mechanisms based on insect-host-plant interaction. Mostly a combination of these 3 mechanisms is present in resistance. These are

- a - Non-preference a variety is less preferred than another variety for oviposition, feeding or shelter
- b - Antibiosis a variety has an adverse effect on the biology of the insect. This is probably the least desirable form of resistance as it places a selection pressure on the insects
- c - Tolerance This is the ability of a plant variety to repair, recover or withstand insect attack. This is the most favorable and natural form of resistance in my opinion, as it does not create selective forces to break resistance plants

and insects in their individual struggle for survival have found a status in which no evolutionary forces to break this status are present. It also permits continuous insect populations as hosts for parasites and predators. It requires however training of the crop grower to convince him that no economic losses will result, within certain limits (the economic threshold population), despite the presence of insects

3.- Literature on resistance in beans to E. kraemer

In the literature, eg Gutierrez et.al. (1975), E. kraemer is reported as the principal bean pest in Latin America. It does not transmit virus diseases but it is suspected that it induces growth regulating substances in the plant tissue. The wide distribution of the leafhopper and its high population numbers, especially in dry season conditions, contribute to its importance.

The literature on E. kraemer is limited, while most research has been done on E. fabae. The two species were separated in 1957 by Ross and Moore and E. fabae seems to be limited to the USA while E. kraemer is reported from Florida, Latin America and the Caribbean. E. kraemer does not transmit virus

diseases in beans. The only leafhopper known to transmit virus diseases in beans in America is the beet leafhopper Circulifer (= Eutettix) tenellus, which transmits curly top. Resistant varieties to the beet leafhopper like Idaho Refugee (reported resistant to E. fabae) Burpee Stringless Greenpod and Landreth Stringless Greenpod do also have reduced virus incidence (Hallock, 1946).

Chalfant (1965) tested 28 varieties for leafhopper (E. fabae) resistance. He found a highly significant positive correlation ($r = 0.64$) between insect counts and damage scores. The number of nymphs per leaf ranged from 0.15 (on Topcrop) to 2.21 (on White Half Runner), while the damage on a 0-10 scale ranged from 0.4 (C 14) to 3.5 for Mountaineer.

McFarlane and Reenan (1943) tested 27 varieties of Ph. vulgaris for E. fabae resistance. They found Henderson Bush Lima most resistant while Idaho Refugee ranked 3rd. The resistance ratings of the same varieties over the 4 replicates was very uniform, indicating homogeneity of infestation.

Most research on resistance in beans to E. fabae has been published by Wolfenbarger and Sacksman in 1961. They also found a highly significant positive correlation between hopperburn rating and nymphal counts ($r = 0.90$), however they also found

tolerance to nymphal attack. This was expressed in FM-52 which had a hopperburn rating of 0, while the nymphal population per leaf reached 15.0. They value resistance scores based on nymphal population higher than hopperburn ratings as this also included preference for oviposition. Low nymphal counts were obtained from PI 151-014 and PI 173 - 024 with 0.3 and 0.4 nymphs per leaf, respectively, while Dutch Brown with 19.7 nymphs per leaf was the highest recorded. They obtained a correlation of $r = -0.19$ (n.s.) for number of epidermal hairs and nymphal population. Therefore hair density was not related to resistance but hair type still may be. In other Phaseolus species they also found great differences in resistance ranking among the varieties. High levels of resistance were found among Ph. aureus and Ph. lunatus and Ph. radiatus sources. They found in interspecific crosses between resistant and susceptible materials indications that resistance was recessive. Plant characteristics that were studied for association with resistance showed, that pod color, leaf area, and growth habit were uncorrelated with nymphal counts, however taller plants had significantly fewer nymphs, and also intermediate maturing varieties and those possessing mosaic resistance were more resistant. Pink seeded varieties were more resistant than those with a mottled seed color.

Medina and Guerra (pers. comm.) found Empoasca resistance, in Negro 66 and Canario 101, which were also resistant to the Mexican bean beetle and the bean pod weevil. Avalos (pers. comm) also found large differences in Empoasca resistance among bean lines, with some lines giving equal yields in protected and non-protected plots

4.- CIAF's Empoasca kraemeri resistance program.

CIAF's entomology program has as main objective to reduce the economic importance of pests by increasing the resistance to these pests in new bean varieties. It is hoped that in this way the need for insecticides will be reduced and that in absence of chemical control measures a reasonable yield can still be obtained.

Development of resistance to Empoasca kraemeri, the principle bean pest of Latin America, is the first objective in our program.

To achieve above objectives for E. kraemeri varieties were screened for resistance to E. kraemeri. First commercial varieties were tested, and as the levels of resistance found were not high enough, resistance sources mentioned in the literature were tested, followed by the screening of the CIAF's germplasm bank (about 8000 have been screened, which is the available

part) Until now we have not encountered levels of resistance high enough to ensure good yields under high leafhopper populations. Some attention is paid to levels of resistance in other crossable species with Ph. vulgaris, like Ph. coccineus. However the main emphasis is placed on a large hybridization program within Ph vulgaris to raise the level of resistance, or to combine different resistance mechanisms to raise resistance in this way.

After screening the available material in the germplasm bank of CIAT, 395 entries were selected for advanced testing. These entries will be planted in replicated plots to score damage, in the form of hopperburn and to make nymphal counts and instar distribution of the nymphs, which we hope will indicate antibiosis type of resistance. Until now, field tolerance was the main type of resistance selected for. Some materials, reported resistant, were not classified among the most resistant entries.

Mechanisms of resistance

Lines selected for resistance in the field did show average leafhopper populations. In detailed laboratory tests with few varieties we found a significant ovipositional non-preference in ICA-711, a black seeded variety being least preferred. But

placing the varieties individually, in cages without choice, no differences were found in oviposition rate, indicating a low level of non-preference. The non-preference was as well for oviposition as for feeding when tested with males only. Antibiosis in these selections and in 54 additional ones tested, was not found.

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1077-1079

THE IMPORTANCE OF EMPOASCA SPECIES
AS BEAN PESTS, TYPES OF INJURY,
THEIR BIOLOGY AND CONTROL

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From a taxonomic standpoint, the genus Empoasca is large and difficult. More than 300 species have been described. They are distributed throughout the world and range from Arctic regions to the Tropics. It seems apparent that many more new species remain to be identified and described. Identification is difficult because specific characteristics for differentiating species are based on the internal male genitalia that are revealed only by clearing the insect in heated, caustic potash (KOH) solution, dissecting in glycerine, and examining them under high magnification (Langlitz 1964). To positively identify an Empoasca female, it is necessary to rear her progeny under isolation and to examine the male offspring.

Distribution of a given species of Empoasca is generally confined to one region although it may be widespread in a continental area. For example, the potato leafhopper, Empoasca fabae (Harris), is widely distributed in the eastern half of the United States and parts of Canada but does not occur in South America as once reported (Langlitz 1964). The southern garden leafhopper (E. solana DeLong) is generally distributed throughout the United States but has not been reported from the Tropics. The economically important leafhopper (Empoasca kraemeri Ross and Moore) is known from Florida, Puerto Rico, Mexico and intervening Central American countries to Peru. In El Salvador, it was apparently the dominant species in a complex with three other species of Empoasca.

Many species of Empoasca in temperate regions live throughout the year in the same general area, the winter is passed as hibernating adults or as eggs in plant tissue. However, the potato leafhopper, Empoasca fabae, does not survive in colder regions but winters only in the warmer Gulf States and migrates northward each spring. From two to three generations develop on crops during the summer growing season. The female inserts two or three eggs per day into the veins or petioles of leaves on the underside. Eggs hatch in about 10 days, and wingless nymphs increase rapidly in size and molt 5 times when they become adults in about two weeks. Populations are highest from June to September after which they decline and disappear with freezing weather.

Types of Feeding Injury by Empoasca

The species of Empoasca may be divided into two groups according to their feeding habits (1) Those that feed on the spongy mesophyll and palisade tissue cause the cell walls to be torn, the cells emptied of their contents, and definite stippling on the upper surface. Of 5 species studied, each caused a stippling distinct for the species. Also, these mesophyll-feeding species excreted dark fecal deposits characteristic for each species, whereas the phloem-feeding non-stippling species excreted clear droplets (Smith and Poos 1931). (2) Those species that feed on the phloem and other vascular tissue deposit sheath material that interferes with translocation which results in accumulation of carbohydrates and in the development of secondary symptoms of yellowing, reddening, distortion, and death of leaves. Wilting and death follow when xylem tissue is plugged and disorganized by the feeding leafhoppers (Smith and Poos 1931). Smith (1933) showed that sheath material in the feeding tracks in Empoasca fabae could be differentiated from plant material by color reactions that indicated it was proteinaceous. Johnson (1934, 1938) was able to reproduce the symptoms of leafhopper feeding in clover and alfalfa by mechanically severing phloem and xylem tissue in the vascular bundles and concluded that carbohydrate accumulation and characteristic symptoms of the affected host were due to clogging of the food-conducting elements and improper translocation.

Medler (1941) in histological studies on feeding punctures by E. fabae noted hypertrophy of affected cells characterized by nuclear enlargement and prominent safranin-staining nucleoli. He interpreted these local changes as evidence of the insect's toxicogenicity as the cause of primary symptoms. External symptoms of chlorosis and reddening as noted by previous workers were ascribed to interference with translocation and considered to be secondary symptoms (Carter 1962). The nature of the toxicogenicity by E. fabae has not been identified. However, Herford (1935) reported that E. solana DeLong, which like fabae feeds in the phloem tissue and causes hopper burn, injects the enzyme diastase while feeding. This enzyme is capable of inverting sucrose.

Crop Damage by Empoasca Leafhoppers

The potato leafhopper, Empoasca fabae (Harris), is the most serious pest of potatoes in the eastern United States and is known to feed on more than 100 cultivated crops and wild plants. The insect overwinters in Florida and other Gulf States where it breeds on alfalfa, beans and weeds. It migrates northward in the spring, feeding on young leaves of oak, hickory and other forest trees. It appears suddenly in May or June on beans, clover, alfalfa, and young shoots of apple, maple and many forest trees.

Later, it attacks potatoes when sugar content becomes higher. Populations can reach 12 to 25 million leafhoppers per hectare. Injured leaves on beans, apples and forest trees are stunted, dwarfed, crinkled and curled. On alfalfa, leaves become yellowed and, on clover, leaves become reddened. Nymphs cause more damage than adults.

The potato leafhopper feeds on the veins on the underside of leaves. On potatoes, where the injury is called "hopper-burn," the first symptom is a brown, triangular patch at the tip of the leaf, followed by similar patches at the end of each lateral veinlet. Entire margins become brown and turn inward until only a small area of green remains along the midvein. Yield of potatoes is greatly reduced.

Empoasca kraemeri (Ross and Moore), another phloem feeder, was described from beans in Nicaragua and has been reported from Columbia, Cuba, Honduras, Mexico, Panama, Puerto Rico and Florida. We found it to be the most abundant species on beans in El Salvador. Langlitz (1964) reported it from Peru on cotton, potatoes, sweet potatoes, barley, beans, corn, Panicum sp., peanuts, Ricinus communis, Vigna sinensis, and tobacco. According to Langlitz (1964), the earlier report that E. fabae (Harris) occurred in Peru was an error. The species involved was actually Empoasca kraemeri. Of the 15 species of Empoasca reported by Langlitz from Peru, 11 species are described as new. Although Langlitz listed 10 species from economic crops in Peru, he did not relate the nature of their injury. His bibliography includes reference to a number of taxonomic papers which should be valuable as reference material.

According to my observations in El Salvador, Empoasca kraemeri in association with three other species, E. prona, E. arator, and E. spp., was most abundant on bean crops during the dry season. Curling and chlorosis of bean leaves and stunting of growth was typical of that caused by Empoasca fabae. In the Sudan, E. libyca de Bergevin, a phloem feeder, caused severe hopper-burn injury on cotton. Cotton-growing in Luzon was prevented because of E. flavescens Fab. that caused wilting and drying of leaves. In Hawaii, E. solana causes severe hopper-burn injury on lettuce and celtnce (Carter 1962).

Among the mesophyll-feeding species of Empoasca, several occur in the United States as major pests of vegetable crops. The intermountain leafhopper E. filamenta DeLong occurs at high altitudes in arid regions where rainfall is under 25 cm and is a pest of beans, sugar beets and potatoes. The western potato leafhopper, E. abrupta DeLong, and the arid leafhopper, E. arida DeLong, occur at low altitudes and low relative humidity from Texas to Oregon and cause extensive stippling of foliage on bean and potato. The apple leafhopper, E. maligna (Walsh) causes extensive stippling injury on apple foliage in the eastern United States (Metcalf 1962).

The cotton leafhopper, Empoasca devastans Distant, is the principal pest of cotton in India and also breeds on other crops (Nielson 1968).

Disease Transmission by Empoasca

The papaya leafhopper, Empoasca papayae Oman, was first suspected in 1938 as a vector of bunchytop virus in papaya in Puerto Rico. Adsuar (1946) reported the successful transmission of this disease in Puerto Rico. The symptoms of the disease were expressed in 71 of 90 trees in about 1-1/2 months after inoculation. Sein and Adsuar (1947) found that males as well as females transmitted the disease. Bird and Adsuar (1952) found additional information on the nature of the virus. This leafhopper is the only known vector of bunchytop virus of papaya.

The cotton leafhopper, Empoasca devastans Distant, is the principal pest of cotton in India and also breeds on hollyhock, castor plant, eggplant, potato and okra. This species was reported as a vector of little leaf of eggplant (Thomas and Krishnaswami 1939). However, only one of 9 plants tested became infected and there are no later records confirming transmission. It is unlikely that this species will become important (Nielson 1968) as a vector although its damage by direct feeding is of great economic importance.

Control of Empoasca

Plant resistance In studies on resistant and susceptible potatoes, varietal differences in reaction to feeding by E. fabae may involve both morphological and physiological characteristics. The resistant variety, Sequoia, compared to the susceptible Cobbler, has thicker-walled collenchyma and its phloem is more extensive and is at a greater distance from the lower epidermis and thereby less accessible to the leafhoppers (Peterson and Granovsky 1950). Sleesman (1940) in tests with 12 species of Solanum found that four species were highly resistant or immune. In tests comparing 27 varieties of snap beans and lima beans for resistance to E. fabae, McFarlane and Rieman (1943) found that early maturing varieties were generally more susceptible than late maturing varieties.

I have made limited observations on beans from the world collection that Ing. Beinado Patinio has grown in El Salvador for testing susceptibility and resistance to both golden mosaic infection and Empoasca injury. Because of the great differences among individual plants in the same lines, it appeared that selections for resistance would be successful. It was noted that at least some resistant plants in these plots had excessive numbers of hooked, spiny hairs on the leaves and stems. In earlier studies, we have found that many leafhopper nymphs become impaled on hairs of this type and that populations did not increase.

Chemical control In the United States, control of E. fabae is essential in the successful culture of potatoes, beans, alfalfa, clover and many other crops including ornamentals such as dahlia, marigold, lupine and tree seedlings

Insecticide applications (DDT dusts and sprays) to alfalfa resulted in 300% yield increase, to beans 80% yield increase (Carter 1962) Soil systemics (thimet and disulfoton) on beans and tree seedlings have been highly effective in protecting the crops for a full growing season in our tests at Beltsville, Maryland

Current registered pesticides for leafhopper and Mexican bean beetle control on beans in New York State and the U S are carbaryl 80 WP (1.12 kg), dimethoate 2.67 EC (28-56 kg), parathion 8 EC (56 kg), Guthion 25 EC (56 kg), all as foliage sprays per hectare, also Disulfoton granules as a systemic in the soil at planting time

In El Salvador experiments initiated in cooperation with Ing. José Mancia (now at the University of El Salvador), both soil systemics and foliage sprays were highly effective against Empoasca kraemer and associated species on beans. In later tests, one to three applications of Azodrin foliage spray provided excellent control and increased yield of beans

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Transmisión del Mosaico Dorado de la Habichuela (Phaseolus vulgaris)
en Puerto Rico por Medios Mecánicos

Julio Bird, Rita Rodríguez, Amelia C. Monllor y Josefina Sánchez¹

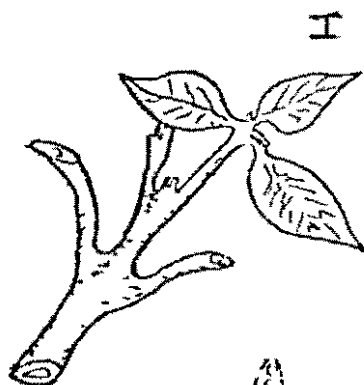
La haba lima (Phaseolus lunatus) es la hospedera primaria del agente que causa el mosaico dorado de la habichuela (P. vulgaris) en Puerto Rico. Este mosaico se propaga con extrema facilidad de plantas enfermas a plantas sanas de las antedichas especies a través de la mosca blanca Bemisia tabaci Genn. Los resultados obtenidos en Puerto Rico en la transmisión de este mosaico por medio de la técnica de Meiners et al utilizando inóculo procedente de P. lunatus no fueron muy halagadores (menos de 18% infección). Por el contrario la proporción de plantas enfermas a sanas aumentó hasta un 25% cuando se utilizó savia sin diluir de las variedades Diablo y Top Crop. Estos últimos resultados se obtuvieron, desde luego, durante la época de verano cuando las temperaturas diurnas en el invernáculo fluctuaron entre los 27° y 35°C. Se lograron resultados de hasta 100% de infección cuando se empleó inóculo procedente de plantas de habichuela Top Crop y Diablo. El proceso usado en la preparación de dicho inóculo fue el siguiente. Se escogió follaje tierno con mosaico bien definido de plantas recién infectadas (14-21 días después de haber sido inoculadas). Para la maceración se usaron morteros y trituradores congelados. La trituración se hizo en presencia de una solución amortiguadora fría de fosfato potásico (0.1 M K_2HPO_4 , pH 7.0) a razón de 1 g de hojas tiernas por 4 ml de solución amortiguadora. La pulpa resultante fue exprimida a través de gasa estéril. Después de añadir 1 g de carborundum (N-600) por cada 100 ml el inóculo se

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virtió en una vasija de cristal rodeada por cubos de hielo. La savia así diluida fue usada directamente para inocular. Se emplearon para este propósito recortes de gasa, hisopos de algodón o pincel de aire. En el caso del pincel de aire el inóculo se coló a través de una capa fina de algodón para evitar la obstrucción del pincel por fragmentos de tejido vegetal. Para efectuar la inoculación por este medio, la presión fue mantenida a 80 lb por pulgada cuadrada y el pistero (boquilla) se abrió 1 1/2 vueltas. Las plantas fueron inoculadas en la etapa de hoja primaria, esto es, antes de que afloraran las primeras trifoliadas. Por medio de este método se consiguió hasta un 100% de infección. La inoculación con el pincel de aire es rápida y permite el manejo-tratamiento de grandes poblaciones en muy poco tiempo. Desde luego que el consumo de inóculo es mayor que el que se utiliza por otros métodos pero no tanto como para que se descarte éste a favor del método de inoculación a mano el cual resulta ser muy tedioso.

Es curioso notar que dos de estos agentes rugaceos se comportan de manera similar en lo referente a su actividad infecciosa sobre el huésped primario al inocular por medios mecánicos. Al inocular mecánicamente con sus respectivos virus se logra un bajo índice de infección tanto en el caso de Euphorbia prunifolia como en el de Phaseolus lunatus. No sucede así cuando se efectúa la inoculación a través del vector natural, i.e., la mosca blanca E. tabaci. Sin embargo, ciertas otras especies como por ejemplo la habichuela en cuanto al mosaico dorado y Datura stramonium en cuanto al mosaico de E. prunifolia, son excelentes huéspedes y fuentes de los referidos virus. Es de notar que esta actividad infecciosa sólo se observa en ciertos y determinados huéspedes como los ya citados. Es muy probable que

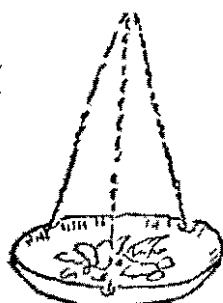
lo mismo suceda con otros virus rugáceos que han demostrado ser altamente refractarios a la transmisión por medios mecánicos. Sabemos ya que con el virus de Rhynchosia minima la savia procedente de plantas de tabaco infectadas es más infecciosa a la habichuela que la que procede del huésped primario Rhynchosia minima. Es muy posible que este fenómeno sea debido a la presencia de sustancias inactivantes que surgen en la savia como resultado de la trituración de los tejidos de ciertas y determinadas plantas huéspedes.



I

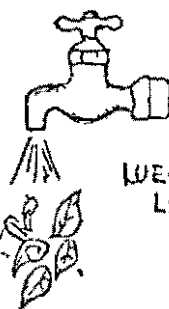
TOMAR HOJAS TIERNAS
DE PLANTAS AFECTADAS
VARIEDAD TOP-CROP
14-21 DIAS DESPUES
DE INOCULACION
NO USAR PLUNATUS COMO FUENTE

II



PESAR HOJAS SECAS

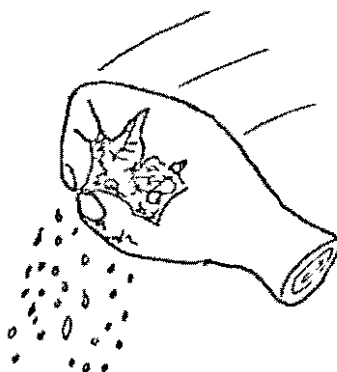
III



LUEGO
LAVARLAS

IV

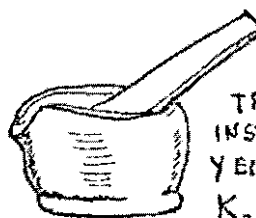
LIBRAR HOJAS DE AGUA
EN EXCESO (SACUDIENDOLAS
BRUSCAMENTE)



V

PESAR HOJAS (MOJADAS)
DE NUEVO

VI A



TRITURAR EN FRIJO CON
INSTUMENTOS CONGELADOS
Y EN PRESENCIA DE 0.1 M
 K_2HPO_4 , PH 7 (FRIA)
PROPORCION 4CC POR
CADA GRAMO DE TEJIDO
FOLIAR

VI B

DIFERENCIA ENTRE PESO HOJAS
SECAS Y MOJADAS SE RESTA DEL
VOLUMEN DE SOLUCIÓN AMORTIGUADORA
QUE HABRÁ DE AÑADIRSE

EJEMPLO: PESO HOJAS SECAS- 10 GRAMOS
PESO HOJAS MOJADAS-13 GRAMOS
DIFERENCIA 3 GRAMOS

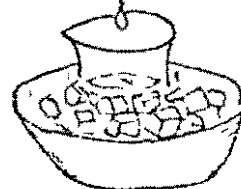
VOLUMEN SOLUCIÓN AMORTIGUADORA A
AÑADIRSE DURANTE TRITURACIÓN -

$$10 \times 4 - 3 = 37 \text{ CC}$$

VII

SE EXPRIME MACERADO
ATRAVES DE GASA ESTERIL Y
DENTRO DE VASIA EN
BAÑO DE HIELO SE AÑADE
1 GRAMO DE CARBONUM* POR
CADA 100CC DE
INOCULO

* 400-600 M



II

SE EMPLEA EL INOCULO DIRECTAMENTE

{ HISOPO ALGODON
GASA
BOLLO DENTAL

E UTILIZA EL PINCEL DE AIRE REGULADO A 80 LIBRAS POR
CADA CUADRADA CON BOQUILLA ABIERTA 1 1/2 VECES

ANTES DE EN INOCULARSE EN ETAPA HOJAS PRIMARIAS Y EN
VERNACULO CON TEMPERATURAS DE VERANO (30-35°C)

Classification of strains of Bean common mosaic virus and genetic interaction between these strains and Phaseolus vulgaris L.

Introduction

The seed borne and aphid transmitted Bean common mosaic virus (BCMV) is confined to beans but world-wide in distribution. It is the incitant of "common mosaic" and "black root disease", the nature and severity of the symptoms greatly depending on variety, virus strain and conditions.

So far, host genetics concerned in resistance to different strains of BCMV is not well understood, while genetics of plant viruses in general is still in its infancy.

Some 18 strains of BCMV have been reported, but most of them have been described on unreliable grounds.

In this study I have tried to improve the knowledge of the host-virus relationship by 1) analysing the reaction types of beans after inoculation with different strains; 2) grouping the varieties according to their resistance spectrum with the Dutch strains, 3) classifying the strains described so far on the basis of their reaction on bean differentials; and 4) studying inheritance of resistance by means of various crosses.

Analysis of host reaction

The host reactions of bean plants on inoculation are shown in table 1.

Table 1

A variety with necrosis gene I recessive may produce mosaic with some strains. Plants with gene I dominant may produce necrosis but never mosaic.

Plants with recessive necrosis gene may have no symptoms at all after inoculation, or only a local defence reaction, or systemic mosaic, sometimes combined with a local defence reaction, depending on virus strain and variety. The sometimes found superficial parenchyma discoloration on the upper surface of the inoculated leaves is indicated as local defence reaction. I consider plants with only local symptoms and no systemic virus spread as resistant, plants with systemic symptoms as susceptible. Thus the plants with recessive necrosis gene without symptoms and those with only a local defence reaction are resistant. Plants with mosaic are susceptible to the strain involved, but may be resistant to other strains.

Varieties with dominant necrosis gene may have no symptoms at all, or may show local pin-point lesions, or local lesions that extend into local vein necrosis, or local vein necrosis followed by systemic necrosis (black root), depending on virus strain and variety.

With some strain-variety combinations, systemic necrosis is induced, practically without local necrosis. Analogous to the division in the plants with recessive necrosis gene, the plants without symptoms or with only local necrosis are considered to be resistant to the strain concerned and the plants with systemic necrosis as being susceptible to that strain.

Thus, in the variety group with recessive and in that with dominant necrosis gene, there is a type of resistance, not showing symptoms with certain strains. However both types can be recognised by inoculating a detached leaf of each plant with a strain that readily induces necrosis (necrosis test). The leaf of a plant with dominant necrosis gene will show pin-point lesions, mostly followed by vein necrosis, the leaf of a plant with recessive necrosis gene not.

Variety testing for resistance spectra

Some hundreds of varieties, breeding lines, P I. numbers and Phaseolus species were tested with six Dutch strains. These strains were: Westlandia, Imuna, Michelite, Great Northern, Jolana and Colana named after the varieties from which they were isolated. As some of these varieties are also used as differentials, the names of the strains are confusing. Therefore I have now encoded them as NL1, NL2, NL3, NL4, NL5 and NL6, respectively.

As a result, the Phaseolus-genotypes could be divided into 11 groups, each with a different resistance-spectrum. In table 2 they are mentioned together with the varieties and breeding lines of each group, used by me or others as differentials.

Table 2

The variety groups 1-6 contain genotypes with recessive necrosis gene, the groups 7-11 genotypes with dominant I. The classification is based on systemic symptoms. Resistant plants show no systemic symptoms, while spread of the virus cannot be proved by indexing to the very sensitive variety Double White (DW).

No varieties with resistance to all strains were found. Only the breeding line IVT7214 (recessive "i") showed resistance to all strains, as did the breeding line IVT7233 (dominant "I"), selected from cross GN31 x ... IVT7214 shows no symptoms at all, while in IVT7233 local pin-

One of the aims of the research, to find genotypes with resistance to all strains, has been achieved with these IVT-breeding lines.

Identification of strains

All strains described up to 1975 were used for comparison, except two German ones, which are no longer available and the Costa Rica and Peru strains, of which no material was sent on request. In addition, two recently found, not yet published, Dutch strains, indicated as NL7 and NL8 were used.

The strains were compared in several tests on the differentials of table 3.

Table 3

In table 3 the virus strains are arranged in seven classes. These classes differ in reaction of the differentials with recessive "i" (host groups 1 to 6). The strains within a class give the same reaction on the varieties with recessive "i", but the reaction on the differentials with dominant "I" may differ. Hence a class may comprise identical strains and "parallel" strains, only differing in reaction on varieties with dominant "I".

In the latter cases the class is subdivided into a and b. The strains in subclass b more readily induce systemic necrosis than those in subclass a.

In fact, strains of one class or subclass are merely isolates of one strain. These investigations show that the Westlandia, Puerto Rico and probably the Costa Rica strain all are isolates of the older Type strain. The Western and Colana strains are isolates of the Idaho strain, while the Mexican strain an isolate is of the Great Northern strain.

The strains Costa Rica and Peru are provisionally placed in classes I and II respectively. Strain Peru in class II together with NL7, originally isolated from plants grown from seeds from Peru.

The results of Table 3 not always agree with data published by the authors of the strains, probably due to original contamination of the strains, genetical impurity of the differentials or contamination of some seeds of the differentials with unknown strains. Obviously "new" strains have also been described with help of incomplete ranges of differentials.

Most observations in Table 3 were simultaneously made in the USA by Dr. Silbernagel as a result of co-operation, using identical virus isolates and seed lots of the differentials.

For proper identification of strains it is essential to use a standard range of differential varieties, increased in insect-free glasshouses

and inspected for diseases to exclude seed infection. As minimum one representative of each host group of Table 3 should be used except the not necessary groups 6 and 11. Back inoculation to a highly sensitive variety such as Double White is a must in cases of no or doubtful systemic symptoms.

Inheritance of the resistance

As not both the strain identification and the inheritance of the resistance can be presented in detail here, only some indications about method and segregation results of the analysis of the inheritance of the resistance are given.

Diallel crosses were made between representatives of host groups 1 to 6. In these 6 differentials with recessive "i" different resistance genes must be present.

The F₁ and F₂ of these combinations were tested with a representative of all strain classes. So the F₁ and F₂ of 15 combinations were tested with 7 strains to analyse the inheritance of resistance and the number of genes involved.

The same resistance genes, or some of them, will be present in the differentials with dominant "I", as these varieties genetically originated from varieties with "i" recessive. These differentials with dominant "I" were crossed with Michelite and GN31.

After determining the resistance genes of the differentials with recessive "i", those of the differentials with "I" dominant could be determined.

Genotypes for resistance and pathogenicity

The results of the analyses of the crosses between differentials with recessive "i" were compared with the expected results after some models of genotypes.

Table 4

Table 4 shows genotype models in which resistance genes form a gene-for-gene relationship with pathogenicity genes. One "a" gene in these models, together with "s" recessive, may give resistance to more than one strain. The recessive alleles of "s" are necessary to obtain resistance to any strain. In Double White the gene is present in dominant condition in the other differentials with recessive necrosis gene, in recessive condition. In table 4 two genotype models are presented, one with the genes a_1 to a_4 on different loci and another with one locus for the "a" genes, a_1 to a_4 and the dominant allele A forming a series of multiple

alleles. "A" dominant gives susceptibility to all strains. For the virus strains, the pathogenicity genes P1 to P4 are supposed to be present in different combinations.

A differential is susceptible if the strain involved has corresponding P genes for all the resistance genes "a" in that variety. The strain has overcome the resistance genes of the variety. But on the other hand, a recessive "a" gene gives resistance to all strains lacking the corresponding P gene.

Up to now the results of the genetic analyses are mostly in agreement with those expected in the case of "a" genes on different loci.

For the line IVT/214 we need an addition to the gene model with a gene "b", together with "s" recessive giving resistance to all strains, no matter which "a" genes are present.

Then the "a" genes of 7214 are not a1 to a4 as indicated in the gene model, but, based on the inheritance analyses, must be A1A2a3A4 as in Michelite.

After determining the resistance genes of the differentials with "i" recessive, those of differentials with dominant "I" will be analysed. As a result the gene formulas of the differentials with recessive "i" could be tentatively designed as shown in table 5.

Table 5

Gene-for-gene relationship and strains to be expected

In table 6 a gene-for-gene relationship is shown between the resistance genes a1 tot a4 and the pathogenicity genes P1 to P4

Table 6

Against 16 host-genotypes 16 virus-genotypes are presented. So far 5 host-genotypes and 7 virus-genotypes have been found

It seems likely that the strains evolve as indicated in figure 1. The development along the line P1, P1P2 is the most productive one. This seems in favour of a theory of evolution to genotypes with genes of higher "order". In the future strains will probably be found in which the genes P2 and P4 are combined for instance P1P2P3P4, attacking all varieties with "i" recessive, except the line IVT/214.

Final remarks

From this study it has become clear that the resistance mechanism of P.

vulgaris to BCMV is based on at least 4 types of genes. We distinguished an assistant gene "a", of which the recessive alleles are necessary for resistance; four "a" genes, of which the recessive alleles form a gene-for-gene relationship with four pathogenicity genes of the strains of BCMV, a gene "b", of which the homozygous recessive alleles confer resistance to all known strains of BCMV, and a necrosis gene "I", preventing mosaic, but governing the development of necrosis after infection with a strain having the ability to induce necrosis. Whether local or systemic necrosis arises depends on which alleles of "a" and "b" are present. For systemic necrosis, besides I dominant, either S dominant, or A dominant or recessive "a" alleles are necessary, overcome by the strain concerned. Evidently "b" has to be present with dominant alleles.

The breeding lines IVT 7233 and IVT 7214 confer complete resistance to all known strains. IVT7233 with genes a1 and a4 gives only pin-point lesions on the inoculated leaves with the easy necrosis inducing strains NL8, NL3 and NL5 and no symptoms at all with the other strains. At present this resistance is sufficient. However a new virus strain combining the genes P3 and P4, will be able to induce systemic necrosis in IVT7233 and to overcome its resistance.

The line IVT/214 may longer resist the evolution of the virus thanks to its gene "b", apparently having a different nature than the "a" genes.

There are still a few questions left for instance it is not yet clear why the parallel strains Florida and FL6, or FL-15 and NL2, or NL3 and NL5 differ in their ability to induce necrosis, while having the same pathogenicity genes. Other virus genes must be involved.

Despite these unanswered questions I hope to have contributed to a more efficient breeding for resistance to BCMV and to a better understanding of the relation between host resistance and virus pathogenicity in general.

October, 1975.

Classification of strains of bean common mosaic virus
and genetic interaction between these strains and
Phaseolus vulgaris L

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Summary

The present study was meant to investigate the genetic relationship between *Pl vulgaris* and BCMV with the aim of giving programs for breeding beans resistant to BCMV a sounder basis. A second aim was to obtain genotypes with mosaic-resistance and necrosis-resistance to all strains of the virus, as preliminary trials revealed that such varieties were not yet available.

First the host reaction was analysed and classified. Then some hundreds of varieties and other accessions were tested with six strains. Based on the test results, the *Phaseolus* genotypes could be divided into eleven groups, each with a different resistance-spectrum. Two groups are represented by an IVT breeding line conferring resistance to all strains. With these lines the second aim of the study was attained.

With representatives of each of eleven variety groups twenty-five virus strains and isolates (nine from the Netherlands and thirteen from abroad) were differentiated and classified. Ten strains could be distinguished, having a different pathogenicity spectrum, the other twelve had to be considered as identical with one of the ten.

Crosses were made between differentials of each host group. F_1 and F_2 of the crosses were tested with representatives of each strain class to analyse the mode of inheritance and the number of genes involved

From this study it has become clear that the resistance mechanism of *P. vulgaris* to BCMV consists of at least four different types of genes. We distinguished a gene "s", of which the recessive alleles are necessary to obtain resistance, genes a_1 to a_4 , of which the recessive alleles form a gene-for-gene relationship with four pathogenicity genes of the strains of BCMV, a gene "b" of which the recessive alleles confer resistance to all known strains of BCMV, and a necrosis gene "I", preventing mosaic, but governing the development of necrosis, after infection with a strain having the ability to induce necrosis. Whether local or systemic necrosis arises, depends on which alleles of "s" and "a" are present.

In the gene-for-gene relationship between host and virus genes the possibility of development of new strains and their influence on the present resistance were discussed.

December 1975

Table 1. Reaction types and principal symptoms after inoculation with BCMV

genotype	reaction	symptoms	symbol
1. recessive necrosis gene n1	resistant	a1 absent, neg in necrosis test	R ⁻
		a2 local defence	Rd
	susceptible	a3 mosaic, whether or not with local defence	Sr
2. dominant necrosis gene N1 or n1	resistant	b1 absent, pos. in necrosis test	R ⁺
		b2 local p1-point necrosis	Rn
	susceptible	b3 local vein necrosis	
		b4 systemic necrosis, whether or not with local vein necrosis	Sr

Table 2 Variety groups with different resistance-spectra found by testing

Variety group and representatives used as differentials		Virus strain					
		ML1	ML6	ML2	ML3	ML5	L4
1	Double White, Str Green Refugee, Common Red Mexican	+	+	+	+	+	+
2	Imuna, Puregold Wax	-	+	+	+	+	+
3	Redlands Greenleaf B, Great Northern 123, GN59	-	+	-	+	+	+
4	Michelite, Sanilac, Pinto 111, Red Mexican 34, GN15	-	-	+	+	+	-
5	Great Northern 31, Red Mexican 35, Monroe, GN16	-	-	-	-	-	+
6	IVT7214	-	-	-	-	-	-
7	Jubila	-	+n	+n	+n	+n	-
8	Topcrop, Improved Tendergreen	-	+n	-	+n	+n	-
9	Idusa	-	-	-	+n	+n	-
10	Amanda	-	-	-	-	+n	-
11	IVT7233	-	-	-	-	-	-

+ = susceptible, systemic mosaic, +n = susceptible, systemic necrosis,

- = resistant, no systemic symptoms, virus spread not recovered by indexing

Host groups		Strain class																
		I				II		III	a	IV b			a V b		a VI b		VII	
		West- landia N1	Type Roo N1	Puerto Rico N2	Costa Rica N2	Peru N2		Flori- da N3	West- ern N5	Idaho br B N6	Colona N6	WY-15 N12	Imuna N12	Niche- lite N3	Jolan- da N5	Mexi- can N4	Great North N4	
Differential variety																		
1	Double White	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	
	Strl Green Refuoco	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
2	Imuna	-	-	-		+	-	+	+	+	-	+	+	+	+	+	+	
	Peruold ax	-	-	-		-	-	-	-	+	-	+	+	+	+	+	-	
3	Redlands Greenleaf 8	-	-	-		-	-	+	+	+	+	-	-	+	+	+	+	
	Great Northern 123	-	-	-		-	-	+	+	+	-	-	-	+	+	+	+	
4	Nichelite	-	-	-		-	+	-	-	-	-	+	+	+	+	-	-	
	Red Mexican J4	-	-	-		-	+	-	-	-	-	+	+	+	+	-	-	
5	Great Northern 31	-	-	-		-	-	-	-	-	-	-	-	-	-	+	+	
	Red Mexican 35	-	-	-		-	-	-	-	-	-	-	-	-	-	+	+	
6	IVT 7214	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	
7	Jubala	-	-	-		-	-	-	+	+	+	-	+	+	+	+	-	
8	Tecocro	-	-	-		-	-	-	+	+	+	-	+	+	+	+	-	
	Improved Tendergreen	-	-	-		-	-	-	+	+	+	-	+	+	+	+	-	
9	Wicusa	-	-	-		-	+	-	-	-	-	-	-	+	+	-	-	
10	Aranda	-	-	-		-	-	-	-	-	-	-	-	-	+	-	-	
11	IVT 7233	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	

+ = Susceptible systemic mosaic +n = susceptible systemic necrosis

± = tolerant systemic symptoms may be absent or very weak systemic virus spread recoverable by indexing

- = resistant no systemic symptoms systemic virus spread not recovered by indexing

1 = Host groups 1 to 6 contain "non-necrosis" differentials groups 7 to 11 "necrosis" differentials

2 = Published data of Gams et al (1970) provisional classification of these strains

3 = N151/50 a few of the plants in the other members

Table 4 . Models of a gene-for-gene relationship after Person in which one gene for differential resistance together with gene s may give resistance to more than one strain

Differential variety	Strain group	I	II	III	IV	V	VI	VII
	Virus strains	NL1	NL7	NL8	NL6 or Flor	NL2 or NY-15	NL5 or NL3	NL4
	P genes	Po	P1	P3	P1P2	P1P3	P1P2P3	P1P2P4
	r genes							
De	SA ₁ A ₂ A ₃ A ₄ ¹ or SA ²	+	+	+	+	+	+	+
Helma	sa ₁ A ₂ A ₃ A ₄ sa ₁		+		+	+	+	+
RGB	sa ₁ a ₂ A ₃ A ₄ sa ₂				+		+	+
Michelite	SA ₁ A ₂ a ₃ A ₄ sa ₃			+		+	+	
EN31	sa ₁ a ₂ A ₃ a ₄ sa ₄							+
NT7214	sa ₁ a ₂ a ₃ a ₄ sa ₅							

1 a₁ gives resistance to the strains lacking P1 etc , only one allele of each gene is mentioned,

2 if the genes form multiple alleles

Table 5 Resistance-genotypes of the differentials
with recessive "1"

1. Double White	SSA ₁ A ₁ Λ ₂ Λ ₂ Λ ₃ Λ ₃ Λ ₄ Λ ₄ BB1d
2. Imuna	ssa ₁ a ₁ Λ ₂ Λ ₂ Λ ₃ Λ ₃ Λ ₄ Λ ₄ BB1d
3. Podlands Greenleaf B	ssa ₁ a ₁ a ₂ a ₂ Λ ₃ Λ ₃ Λ ₄ Λ ₄ BB1d
4. Michelite	ssΛ ₁ Λ ₁ Λ ₂ Λ ₂ a ₃ a ₃ Λ ₄ Λ ₄ BB1d
5. Great Northern U I 31	ssa ₁ a ₁ Λ ₂ Λ ₂ Λ ₃ Λ ₃ a ₄ a ₄ BB1d
6. IVT 7214	ssΛ ₁ Λ ₁ Λ ₂ Λ ₂ a ₃ a ₃ Λ ₄ Λ ₄ bb1d

Table 6. Tests for gene relationships between 4 resistance and 4 pathogenicity genes

Possible differen- tials	a' genes	Observed differen- tials	Possible virus strains with pathogenicity genes observed strains																Number of attack- ing strains	Number of genes with resist- ance
			V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16		
				P1	P2	P3	P4	P1 P2	P1 P3	P1 P4	P2 P3	P2 P4	P3 P4	P1 P2 P3	P1 P2 P4	P1 P2 P3 P4	P2 P3 P4	P1 P2 P3 P4		
			VL1	VL7		VL6		VL5 Flor	P2 P3 P4					VL3 NL2	VL4					
1		Double w	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16	0
2	a ₁	Inuma		+				+	+	+				+	+	+		+	8	1
3	a ₂							+			+	+		+	+		+	+	8	1
4	a ₃	Michelite				+				+	+		+	+		+	+	+	8	1
5	a ₄						+			+		+	+		+	+	+	+	8	1
6	a ₁ a ₂	Redl Gr B						+						+	+			+	4	2
7	a ₁ a ₃								+					+		+		+	4	2
8	a ₁ a ₄	GV U 1 31								+					+	+		+	4	2
9	a ₂ a ₃										+			+			+	+	4	2
10	a ₂ a ₄											+			+		+	+	4	2
11	a ₃ a ₄												+			+	+	+	4	2
12	a ₁ a ₂ a ₃													+				+	2	3
13	a ₁ a ₂ a ₄														+			+	2	3
14	a ₁ a ₃ a ₄															+		+	2	3
15	a ₂ a ₃ a ₄																+	+	2	3
16	a ₁ a ₂ a ₃ a ₄																	+	1	4
Number of attacked differ- entials			1	2	2	2	2		4	4	4	4	4	4	4	8	8	8	8	16
Number of gene with pa- thogenicity			0	1	1	1	1		2	2	2	2	2	2	2	3	3	3	3	4

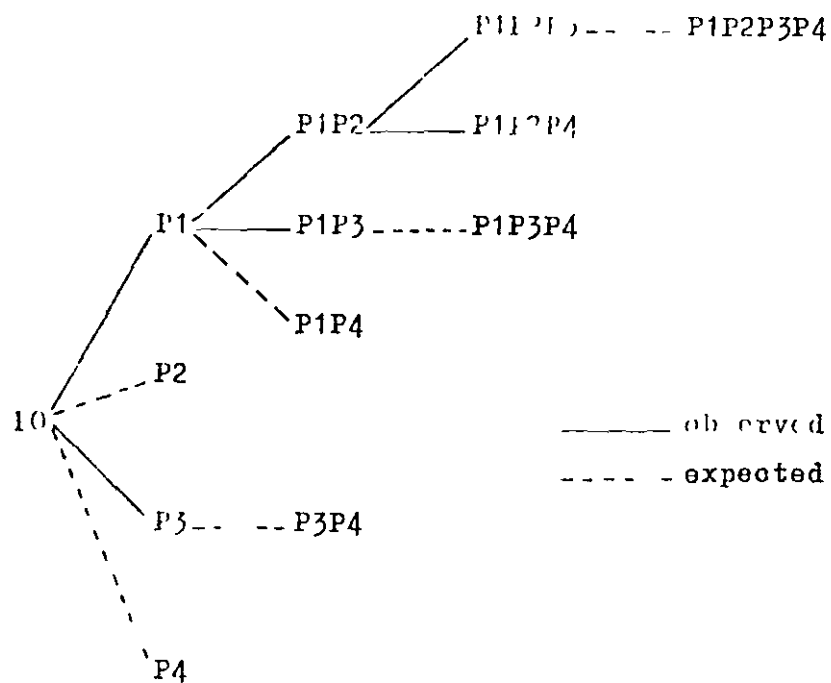


Figure 1 Observed and expected strains of BCIV.

PRINCIPALES ENFERMEDADES DEL FRIJOL (Phaseolus vulgaris) EN LOS TROPICOS AMERICANOS Y SU DISTRIBUCION EN DIFERENTES ZONAS ECOLOGICAS

Eddie Echandi^{1/}

El frijol (Phaseolus vulgaris) y unas 126 especies del género Phaseolus son de origen Americano P. vulgaris se originó en Mexico y Guatemala (13)

El frijol ha constituido la principal fuente de proteína de los pueblos de la America tropical, pero a pesar de su importancia los promedios de rendimiento por area siempre han sido bajos En los últimos 20 anos por ejemplo, los rendimientos han sido de aproximadamente 600 kg/ha , y se han mantenido practicamente iguales a traves de los 20 años (6), mientras que otros cultivos como maíz, arroz, etc , han logrado aumentos espectaculares Por otro lado, la población americana consumidora de frijol ha incrementado considerablemente Este incremento de población ha ocasionado un aumento en la demanda de frijol que muchos paises no han podido satisfacer En Costa Rica, por ejemplo, a pesar del aumento de precio del grano el problema de la escasez de frijol se torna mas grave cada ano

Cuales son los factores limitantes de la producción de frijol?, La mayoría de los investigadores concuerdan en que las enfermedades constituyen uno de los factores limitantes de la producción de frijol, y posiblemente las hurgosas sean las que causan los mayores danos (12)

Desde el punto de vista ecológico los trópicos Americanos son sumamente variados y complejos, sin embargo existen mapas ecológicos de paises tales como Costa Rica, Guatemala, El Salvador, Panamá, Bolivia, Puerto Rico, Perú

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y Colombia. Estos mapas permiten identificar y delimitar cartográficamente la relación entre el clima (macroclima) y las formaciones vegetales (14)

El mapa ecológico de Costa Rica fué empleado por Echandi (11) para iniciar estudios de la relación del clima y las enfermedades del frijol. Las características de las cinco zonas ecológicas en que se siembra frijol en Costa Rica aparecen en el Cuadro 1. Los trabajos iniciados en Costa Rica, fueron extendidos luego al resto de los países de Centro America y Panamá. En esta etapa participaron varios técnicos y se colectaron datos por varios años. Desafortunadamente esa información no ha llegado a publicarse.

PRINCIPALES ENFERMEDADES DEL FRIJOL

Roya. La roya causada por Uromyces phaseoli (Reben) Wint. se encuentra ampliamente distribuida en los trópicos americanos y ha sido considerada de primordial importancia en Perú, Brazil, Colombia, México y Venezuela. Los mayores daños al menos en Costa Rica, se producen en las zonas subtropicales y montano bajo (Cuadro 2), la roya no ha causado daños de importancia en los climas secos y muy cálidos de Costa Rica (11). En el trópico el estado perfecto de U. phaseoli aparece en los climas fríos al final del ciclo de la planta. En Costa Rica Christen y Echandi (9), identificaron las razas 3, 8, 10, 15, 22, 25 y 32. En México Crispín y Dongo (7) identificaron 31 razas del hongo. En Colombia Zuñiga de Rodriguez (22) identificó 11 razas. En el Brazil Agustín y Costa (3) identifican cada año las razas predominantes en el Sur de Brazil. En la zona templada (EEUU) se han identificado alrededor de 35 razas.

Christen y Echandi (9) encontraron 6 introducciones resistentes a las razas predominantes en Costa Rica. En México existen varios cultivares resistentes (8). En los EEUU los cultivares US Pinto 5 y 14 son resistentes a muchas de las razas de roya que aparecen en ese país. No existe ningún cultivar resistente a todas las razas conocidas de roya (18).

Antracnosis La Antracnosis causada por Colletotrichum lindemuthianum (Sacc & Magn) Briosi & Cavara es de importancia primordial en Guatemala, México, Brazil, Colombia y Venezuela. En Costa Rica aparece en las zonas subtropicales y montano bajo, no aparece en zonas secas y muy cálidos como la zona tropical (Cuadro 2). Posiblemente esto se deba a que la alta temperatura que predomina en esa zona interfiere con el crecimiento y la esporulación del hongo (17). En estudios conducidos por Villao (20), en Costa Rica se determinó que en ese país predominan las razas alfa beta y gama. Las razas alfa y gama fueron las de más amplia distribución. Olieri et al (16) determinó 7 razas de C. lindemuthianum en Brazil. Villao (20) inoculó 450 introducciones con las razas alfa, beta, gama y delta, y encontró varias resistentes a las cuatro razas. Olieri et al (16) encontró que Cornell 49-242 es resistente a las 7 razas del hongo observadas en Brazil. En México existen 6 o más cultivos resistentes a la antracnosis.

Hace algunos años la Antracnosis era una enfermedad de gran importancia económica en los EEUU. En aquella época la mayoría de la semilla de frijol que se utilizaba en ese país era producida en el Este y el Sur, en donde las condiciones son ideales para el desarrollo de la antracnosis. Hoy la antracnosis no es una enfermedad de importancia en los EEUU, ya que en ese país se utiliza semilla libre de antracnosis y variedades resistentes.

Chasparria La Chasparria es causada por Thanatephorus cucumeris (Frank Donk) Contro por colletotia (Matz) Weber. Esta enfermedad está ampliamente distribuida en los trópicos americanos y causa los mayores danos en climas cálidos y húmedos con períodos prolongados de lluvia (Cuadro 2). Los daños causados por la Chasparria y su aparición súbita, recuerdan al Tizón tardío de la papa, bien conocido por todos los Fitopatólogos (11). Cuando el inóculo es acarreado por la semilla el hongo se disemina rápidamente, ya que

predominan las formas esporulantes y la diseminación por basidiosporas es muy eficiente (10)

En Florida la Chasparria causó daños serios hace algunos años, pero el uso de semilla libre del hongo, rotaciones prolongadas y la siembra de frijol en zonas no propicias a esta enfermedad, son aparentemente las principales razones por las cuales la chasparria no es de importancia económica en los EEUU

Mancha angular La Mancha angular causada por Isariopsis griseola Sacc está ampliamente distribuida en los trópicos Americanos, la enfermedad es seria en El Salvador, Guatemala, México, Colombia, Brazil y Venezuela. En Costa Rica causa los mayores danos en la zona subtropical (Cuadro 2). Silvera (19) encontró 14 líneas de frijol resistentes. Olave (15) encontró que los cultivares México 11, México 12 y Cauca 27a, muestran resistencia. Brock (15) determinó 19 cultivares resistentes y 11 que calificó como muy resistentes.

Mildiu u Oidium El Mildiu u Oidium es causado por Erysiphe polygoni D C ex Haret, aparece en cualquier zona, siempre y cuando las condiciones de humedad sean adecuadas para su desarrollo (Cuadro 2). Se ha observado en Costa Rica principalmente en las siembras de verano (época seca). En general los danos causados por el Mildiu no son muy serios. Sin embargo, en el Perú esta enfermedad puede ser grave (4). Existen cultivares resistentes a varias razas del hongo (21).

Sclerotinia La Sclerotinia causada por Whetzelinia sclerotiorum Karb & Duranont es una enfermedad seria pero afortunadamente de distribución muy restringida, se ha observado en las zonas Montano bajo húmedo y muy húmedo (Cuadro 2). La enfermedad aparece por lo tanto, en lugares altos y fríos sobre todo en épocas de mucha precipitación. Adams (1) determinó 9 culti-

vares de P. coccineus tolerantes y 2 cultivares y 2 líneas de P. phaseolus también tolerantes. Anderson (2) et al encontró 5 cultivares de P. phaseolus tolerantes a Sclerotinia.

Maya blanca La maya blanca causada por Sclerotium rolfsii (Cruz) West aparece en la zona tropical seca y con menor frecuencia en la subtropical húmeda y muy húmeda (Cuadro 2). Es una enfermedad de lugares cálidos. La Maya blanca se presenta amenudo en lugares en que previamente se había sembrado arroz o sorgo. No existen hasta el momento cultivares resistentes. En los EEUU la Maya blanca es importante en las costas Este y Oeste en donde los suelos están infestados con S. rolfsii.

Mancha ascochyta La Mancha ascochyta es provocada por Ascochyta blight Sacc. Aparece en Costa Rica en la zona montana bajo muy húmeda (Cuadro 2). En Guatemala se la encuentra en las montañas altas y húmedas. Esta enfermedad es típica de lugares fríos ya que el óptimo para su desarrollo es alrededor de 20°C. Su distribución limitada en cuanto a clima, posiblemente se deba a la temperatura y en menor grado a la precipitación. No existe información respecto a fuentes de resistencia.

CONCLUSIONES DERIVADAS DE LOS ESTUDIOS ECOLOGICOS

A continuación trataré de enumerar algunas de las conclusiones obtenidas en los estudios ecológicos realizados en Costa Rica y Centro America.

- 1 Los mapas ecológicos son un instrumento valioso para el estudio de las enfermedades del frijol con respecto al clima.
- 2 Existe gran variación en la distribución de las principales enfermedades del frijol en cuanto al clima.
- 3 Muchas enfermedades no se presentan en ciertos climas.

- 4 El sistema permite predecir la presencia o ausencia de ciertas enfermedades en ciertos climas
5. El sistema permite determinar zonas propicias para el cultivo del frijol, así como también aquellas que no lo son debido a la incidencia de ciertas enfermedades

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LAS ENFERMEDADES VIRALES COMO FACTORES LIMITANTES EN LA PRODUCCIÓN

DEL FRÍJOL (Phaseolus vulgaris) EN AMÉRICA LATINA

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INTRODUCCIÓN

Es un hecho claramente establecido que las enfermedades constituyen uno de los factores de mayor importancia que limitan la producción del frijol en América Latina. Numerosas investigaciones han permitido elucidar que patógenos tales como hongos, bacterias, virus y nemátodos son los responsables de un número apreciable de enfermedades que afectan a este cultivo a través de todo Latinoamérica, ocasionando serias pérdidas en su producción. Las enfermedades fungosas son quizás las más numerosas, y entre ellas se hallan la mayoría de las más importantes del cultivo. Las enfermedades virales ocupan un segundo lugar en cuanto a número, pero incluyen entre ellas también algunas de las de mayor importancia económica. Las enfermedades bacteriales y las causadas por nemátodos, son menos numerosas en general que las anteriores.

Antes de referirnos al problema de las enfermedades virales, es conveniente tener en mente el principio clásico de que el desarrollo de una enfermedad está determinado por la presencia del patógeno, de un genotipo susceptible a éste y de una condición ambiental determinada que favorezca al desarrollo y al establecimiento de la relación patógeno-hospedante. Conocemos que el frijol es cultivado en América Latina desde hace muchos siglos,

bajo condiciones ecológicas sumamente diversas, de lo cual entre otras cosas, ha resultado la selección y desarrollo de una enorme variedad de genotipos cultivados, con características también muy diferentes. Es así como podemos encontrar gran diversidad en la identidad, severidad, distribución de las enfermedades de una localidad o región a otra, así como de una determinada época del año a la otra.

Los virus constituyen un grupo de patógenos de características biológicas muy particulares. Entre otras cosas, la sintomatología de las enfermedades que producen, con mucha frecuencia no permite precisar con alguna certeza la identidad del virus involucrado. Así que su identificación correcta requiere de la aplicación de cierta metodología específica, no siempre al alcance de algunos de nuestros investigadores. Encontramos por lo tanto que en la literatura sobre enfermedades del frijol en América Latina aparecen numerosas menciones a enfermedades virales con sólo una descripción de sus síntomas, lo cual no nos permite conocer la identidad real de la enfermedad, pero sí tener una idea de la distribución e importancia relativa de tales enfermedades.

IDENTIDAD Y DISTRIBUCIÓN DE LOS VIRUS DEL FRIJOL EN

AMÉRICA LATINA

Los numerosos estudios realizados en varios de nuestros países, en que se han identificado apropiadamente los virus y las enfermedades que

ellos causan, hace posible ya tener un panorama más preciso de su identidad y distribución en América Latina. En el Cuadro 1 aparecen los principales virus identificados en diversos países de la región.

El virus del mosaico común se encuentra distribuido por toda América Latina. La variabilidad del virus es notoria, ya que tanto en Centro América como en Sur América se han aislado numerosas variantes o razas del virus, que han sido identificadas principalmente en base a la reacción de variedades diferenciales de frijol. La uniformización de criterios para la identificación de razas del virus en América Latina es un aspecto de singular importancia no sólo para propósitos de diagnóstico sino también para la utilización de genotipos resistentes. El virus del mosaico amarillo, muy cercanamente relacionado al del mosaico común, ha sido descrito en la Argentina, Chile, Brasil, Colombia, México y los Estados Unidos.

Bajo el nombre de virus del mosaico rugoso se ha incluido un grupo de virus cercanamente relacionados, o razas de un mismo virus, halladas hasta el momento en Centro América y en el sur de los Estados Unidos. El mosaico surco ha sido identificado en México y Costa Rica, al igual que en los Estados Unidos, el moteado amarillo únicamente en estos dos últimos países.

El nombre del "virus del mosaico dorado" identifica también a otro grupo o complejo de virus cercanamente relacionados o razas de un mismo

virus, descritas en el Caribe, Centro América, Brasil, Colombia y Venezuela. Igualmente, bajo la denominación de virus de la clorosis infecciosa de las malvaceas y virus del mosaico de las euforbiaceas se ha agrupado un complejo de virus originalmente identificados como patógenos en plantas de las familias mencionadas, pero que también infectan el frijol en las mismas regiones que el mosaico dorado. Existen otros virus del frijol de una distribución que pareciera en este momento ser más localizada. Por ejemplo, únicamente en Venezuela se ha informado de la presencia del virus del mosaico de la alfalfa y del virus de la mancha anular del tabaco, en Brasil, del virus del mosaico necrótico / del "vira-cabuca", arborescentes al grupo del "spotted wilt", y del virus "no-vermelho" ('tobacco streak')

IMPORTANCIA DE LOS VIRUS DEL FRIJOL

De los virus actualmente conocidos, es obvio que el del mosaico común es el que posee una distribución geográfica más amplia, lo cual posiblemente sea debido en parte a su transmisibilidad por semilla y por ciertas especies de áfidos como Myzus persicae, que tienen también un rango muy amplio de distribución y adaptación ecológica. Por otra parte, la importancia económica y grado de diseminación local del virus está íntimamente ligada a los genotipos de frijol utilizados preferentemente en la región. En las plantaciones de la costa del Pacífico de Chile y Perú alcanza grados de incidencia hasta de un 100%, causando serias pérdidas en producción de

LOS INSECTOS COMO VECTORES DE VIRUS DEL FRIJOL

El papel de notoria importancia desempeñado por los insectos en la diseminación de los virus del frijol había sido señalado anteriormente. Podemos apreciar claramente, de lo anteriormente descrito, la existencia de tres grandes grupos de virus, de acuerdo al tipo de vector que poseen. Estos son los transmitidos por áfidos, por coleópteros y por moscas blancas. Los transmitidos por áfidos, Myzus persicae principalmente, comprenden los virus del mosaico común, mosaico amarillo y mosaico de la alfalfa. Los transmitidos por Coleópteros, Diabrotica sp y Cerotoma sp entre otros, incluyen el grupo del mosaico rugoso, mosaico sureño y moteado amarillo. La mosca blanca Bemisia tabaci transmite el complejo de virus del mosaico dorado, y los de la clorosis infecciosa de las milvaceas y el mosaico de las euforbiáceas. Mientras que la distribución ecológica de los áfidos es más amplia, los coleópteros y particularmente las moscas blancas y sus respectivos virus parecen estar circunscritos principalmente a las áreas subtropicales y tropicales del continente americano.

OBSERVACIONES SOBRE EL CONTROL DE LAS ENFERMEDADES VIRALES DEL FRIJOL EN AMÉRICA LATINA

Conociendo a grandes rasgos la naturaleza, identidad e importancia de los problemas virales en frijol en América Latina, es adecuado hacer algunas consideraciones generales sobre el aspecto del control de tales enfermedades. La utilización de semilla sana es una de las medidas más sen-

cillas y fáciles de implementar, pero a la que todavía no se le ha dado en nuestros países la importancia que merece. Las ventajas de la utilización de este tipo de semilla son ampliamente conocidas, en lo relativo a virus, daría una solución adecuada para el problema del mosaico común. Es un hecho establecido que la mayoría de las variedades susceptibles a este virus utilizadas en nuestros países, lo transmiten a través de sus semillas en porcentajes notablemente elevados. El inóculo primario proviene casi en su totalidad de semillas contaminadas. Consecuentemente, el uso de semilla sana sería de gran utilidad práctica.

El uso de genotipos resistentes, por otra parte, constituye la medida más efectiva y económica de control de patógenos, incluyendo los virus. Existen numerosas fuentes de resistencia para el mosaico común y mosaico rugoso, que podrían utilizarse en los programas de mejoramiento. La búsqueda de materiales resistentes al complejo de virus transmitidos por moscas blancas requiere atención especial, no sólo por la importancia del problema, sino por la complejidad de la naturaleza viral del mismo.

El aspecto ecológico ha recibido en general poca atención en nuestros trabajos. Un conocimiento adecuado de las condiciones ecológicas requeridas para la sobrevivencia y diseminación de los virus y sus vectores podría darnos no sólo un mejor conocimiento de las enfermedades virales, sino también información relevante al desarrollo de métodos más adecuados para su control.

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CUADRO 1 PRINCIPALES VIRUS DEL FRIJOL EN AMERICA LATINA

PAIS	VIRUS							
	VnC	VMA	VNR	VIS	VMoA	VMD	VCII	VIE
SUR AMERICA								
Argentina		+						
Chile		+						
Perú	+	+						
Brasil	+			+		+	+	+
Venezuela	+					+	+	
Colombia	+					+		
CENTRO AMERICA								
Costa Rica	+		+	+	+	+	+	+
Nicaragua	+		+			+	+	
Honduras	+		+			+	+	
El Salvador	+		+			+	+	
Guatemala	+		+			+	+	
CARIBE								
Puerto Rico	+					+	+	+
Jamaica	+					+		
República Dominicana	+							
NORTE AMERICA								
México	+	+		+				
Estados Unidos	+	+	+	+	+			

VnC Virus del mosaico común, VMA Virus del mosaico anarillo, VNR Virus del mosaico rugoso, VIS Virus del mosaico sureño, VMoA Virus del moteado amarillo, VMD Virus del mosaico dorado, VCII Virus de la clorosis infecciosa de las leguminosas, VIE Virus del mosaico de las euforbiáceas

Se incluye por comparación

MORPHOLOGY OF THE VIRUSES AFFECTING BEAN AND THE ULTRASTRUCTURE
OF THE INFECTED CELLS

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Viruses might, under certain circumstances, cause considerable losses in the bean productivity. The knowledge of the particle morphology and the cytology of the infected cells help to complete the characterization of the causal virus and to understand the basic events involved in the infection process. These informations, furthermore, serve as an aid for a quick and precise diagnosis, an important factor in the control of the diseases caused by these viruses. A brief survey of what is presently known on the morphology and cytopathology of the bean viruses will be presented in the following lines.

ELONGATED VIRUSES

Bean common mosaic (BCMV) and bean yellow mosaic (BYMV) viruses:
These viruses belong to the so-called POTV group (1), with reported normal length of 750nm (2). Particle length up to 800nm has been reported but such variation is probably due to preparative processes (3). BCMV and BYMV are serologically related, but the cytology of the infected cells is somewhat different. In BCMV-infected cells, the lamellar inclusions tend to have a tubular or scroll shape (pinwheels), while in the case of BYMV, these lamellae tend to aggregate in the form of thick flat plates (3-9). A particularly severe strain of BYMV ("Paracriaba") is able to induce in the infected cells, besides the pinwheels, the formation of membranous whorls, apparently derived from the Golgi bodies (10). Both BCMV and BYMV particles can be detected in the cytoplasm of infected cells, either forming aggregates or associated to the lamellar inclusions (4,8).

ISOMETRIC VIRUS

Southern bean mosaic virus (SBMV): SBMV is a very stable, isometric virus (ca 30nm in diam) (11). Virions were detected in thin sections of infected cells, forming discrete aggregates, in the nucleus, cytoplasm, and vacuole (12-14). Large crystalline aggregates of virus particles were observed in the nucleus and cytoplasm of the "coccinea" strain of the SBMV (14).

Line pattern mosaic virus (BIPMV): An isometric virus, ca. 30nm in diam. was isolated in Campinas and named BIPMV for the pattern it caused in bean (15). It occurs in high concentration in infected leaves, being quite easily purified. Infected cells usually contain large crystalline aggregates of the virions in the cytoplasm. Another crystalline inclusion of unknown nature also occurs in these cells. Extensive vesiculation of the cytoplasm is associated with the infection (16).

Bean pod mottle virus (BPMV): This is a virus with bipartite genome, belonging to the group of cowpea mosaic virus (CPMV), with which BPMV is serologically related (17). BPMV is about 30nm in diam. and it has been detected in the cytoplasm and vacuole of infected cells. A fibrous inclusion was occasionally found in the cytoplasm of BPMV-infected cells. A very characteristic feature of the BPMV-infection is the appearance of tubules containing virus particles embedded in the cell wall, often associated with fingerlike protuberances of the latter (18,19).

Bean rugose mosaic virus (BRMV): An isometric (ca. 30nm in diam.) virus, transmitted by chrysomelid beetles, was found in Guatemala; it was named BRMV. It is serologically related to both BPMV and CPMV (20). Tissues infected by 2 strains of BRMV, respectively A1 and A2, were compared in the electron microscope. Cell changes induced by strain A1 was similar to that caused by BLPMV and SBIV (large virus crystals and a crystalline inclusion of unknown nature), whereas infection by strain A2 resembled that of BPMV and CPMV (virions in the vacuole and vesicles, fibrous inclusions in the cytoplasm, proliferation of cytoplasmic vesicles). Outgrowth of the cell wall, containing tubules with virus particles, similar to that described in BPMV-infected cells, was observed only with strain A1 (21).

Bean "ampollado" virus (BAV): This is an isometric virus (ca. 30nm in diam.) found in Central America. Electron microscopic examination of BAV-infected tissues revealed virions in the vacuole, and occasionally in the cytoplasm, forming discrete aggregates near the plastid. Cytoplasmic areas rich in vesicles were commonly observed. In a few cases, virions were noticed within the vesicles (22).

Bean red knot This condition is caused by the Brazilian tobacco streak virus, an isometric virus of ca. 30nm in diameter, and included in the recently created ILAV virus group (15,23,24), with tripartite genome (25).

Cucumber mosaic virus (CMV): A case of field infection of bean by CMV was reported in the Parana State, Brazil (26). CMV is also a virus with tripartite genome (27).

Bean golden mosaic virus (BGMV): BGMV is a whitefly transmitted virus (28). Recent work showed that BGMV-particles are very small, ca. 15nm in diameter, usually occurring as dimers (29). In thin sections, viruslike particles were occasionally seen in the sieve tube, but this needs further confirmation (30). Dramatic change in the chloroplast morphology is induced by BGMV-infection, particularly in its lamellar system (30). In Brazil, the other whitefly-transmitted viruses affect field beans, respectively, infectious chlorosis of calceola, inducing dwarf mosaic, and Euphorbia mosaic, which causes leaf curl. No information regarding the morphology of these viruses is available yet (15).

BEAN NON-SPOTTED VIRUS

Bean necrotic mosaic virus (BNMV): BNV probably belongs to the tomato spotted wilt virus group (15). Its particles are membrane-bounded, 80-100nm in diam., being easily detected in leaf dip preparations. Serological relationship to TSWV was not investigated yet. Virus particles occur within the endoplasmic reticulum of infected cells. A dense, amorphous mass, usually occurs in the cytoplasm of the infected cells (31).

A possibly related virus associated with mosaic and blister: In bean leaf samples collected at Ibadan, Nigeria, rhabdoviruslike particles were found in the nuclear space of cells from leaves showing mosaic and blister. Besides these particles, pinwheel inclusions, typical of infection by IRTV viruses were also noticed (32).

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ELECTRON MICROSCOPY OF MYCOPLASMA- AND RICKETTSIA-LIKE ORGANISMS IN LATIN AMERICA

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Electron microscopy, antibiotic sensitivity tests, and in vitro culture of the agent have demonstrated that several plant diseases, so far considered as being of viral etiology, were caused either by mycoplasma- (MLO) or by rickettsia-like organisms (RLO). In the Latin American countries an increasing number of plant diseases have been associated with MLO or RLO, and most of them were already studied in the electron microscope, revealing the morphology, intracellular localization, and cytopathic effects of the pathogen. Such investigations are important also, because, in many cases, electron microscopy is the only way to indicate the association of MLO or RLO with a given plant disease. A brief survey of these works will be presented in the following lines:

RICKETTSIA-LIKE (POTVIRUS)

Fernandez-Villaverde and Nakao (1) described a graft-transmissible disease of plum, characterized by leaf scalding, at the Delta del Paraná, Argentina. Electron microscopical work demonstrated the association of RLO, present in the xylem, with this condition (2). RLO differ from MLO because they have a characteristically corrugated cell wall, while MLO do not possess cell wall, and consistently occur in the phloem vessels, and not in the xylem. Hirumi et al (3) found RLO associated with little leaf disease of Sida cordifolia, in Puerto Rico. In Chile, Urbina-Vidal (4) claimed to have found RLO together with MLO in sugar beet yellow wilt-affected beet plants. These RLO occurred in vessels associated with plasmodesmata in phloem parenchyma cells. Supermicrotome staining agent might be RLO (5), since bacterioid organisms were found in xylem in samples collected at Puerto Rico.

MYCOPLASMA-LIKE ORGANISMS

Corn stunt agent is presently regarded distinct from mycoplasma, and grouped as mycoplasma (6) together with citrus stubborn agent, due to the characteristic helical form of the cell. It has been described in several Latin American countries, and electronmicroscopical work have indeed revealed the association of mycoplasma with the disease (7,8).

A wilted form of corn in Brazil could be associated with MLO (9), and a similar condition found in corn at Tapachula, Mexico (10).

Yellowing, stunting, and wilted broom symptoms in several ornamental plants. Conocarpus Callistegia chinensis, Chrysanthemum parthenum, Agave variegata, Albizia leucacantha, Cochlospermum fruticosum (11,12). Tobacco variegation (13) - very closely related

Among vegetables, Brazilian tomato big bud, also transmissible by grafting to other Solanaceae hosts, was found associated with MLO (14). Under experimental conditions, a disease broom of Trigeron bonariensis, a compositae weed, was transmitted by tissue union to tomato, in which the agent induced big bud (15). Another case of association of big bud with the presence of MLO in the phloem was observed with the New Zealand spinach (Tetragonia expansa-Asteraceae) (11).

MLO was found associated with witches' broom symptoms of several wild plants (Malvaceae-Sida coriifolia, Malvaceae-Triumfetta parviflora, Sterculiaceae-Valtheria indica; Compositae-Solidago serotina, Leguminosae-Cassia occidentalis), in the São Paulo State (11).

Several leguminous crops of the collection of IPI, Batão, SP, such as Phaseolus atropurpureus, Crotalaria juncea, C. paulina, Desmodium sp. and Glycine sp., were found exhibiting proliferation and little leaf symptoms. Electron microscopy revealed consistently the occurrence of MLO in the sieve tubes of affected plants (16). In Brazil, a witches' broom of bean, transmissible by grafting, was also found associated with MLO (17). In Puerto Rico, Mera orchard et al. (18) observed MLO in the phloem of pigeon pea (Cajanus cajan) with witches' broom symptoms.

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Breeding Dry Edible Beans (Phaseolus vulgaris L) for
Tolerance to Xanthomonas Bacterial Blights

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Seed-transmitted bacterial diseases continue to be serious production-limiting factors for dry beans in many areas of Latin America, as well as in the humid Great Lakes regions of the U S and Canada. Especially important are the diseases incited by Xanthomonas phaseoli (common blight = Xp), X phaseoli var fuscans (fuscous blight = Xpf), and X vignicola (tropical halo blight = Xv). For purposes of simplicity and to avoid confusion, Xp as mentioned in this paper includes Xv.

The 1974 8th Edition of Bergey's Manual designates Xp, Xpf, and Xv as nomenclotypes of X campestris.

Primary sources of infection in Xanthomonas blights include internally- and externally-contaminated seed and overwintered inoculum in the field. Subsequent disease development and secondary spread are favored by humid or wet, and warm weather conditions. Leaf and pod infection can occur through natural openings such as stomata and hydathodes, or through wounds caused by insects, blowing soil particles, machinery, or animals including man. Internal infection of developing bean seed most commonly results from a systemic invasion of the vascular elements of the upper pod suture.

Although practical short-term control of the Xanthomonas diseases is possible through the use of disease-free seed, crop rotation, and possibly chemical sprays, long-term control depends on the development of disease-resistant or tolerant cultivars. It has only happened in the past 10 to 15 years that major programs of breeding Xanthomonas tolerance into dry edible bean types have been initiated. Programs of this type are located at the University of Nebraska (D. P. Coyne and M. L. Schuster), Michigan State University - USDA (M. W. Adams and A. W. Saettler), Puerto Rico - USDA (G. F. Freytag and N. G. Vakili), Canada (J. W. Aylesworth and J. H. Haas), CIAT (G. H. Bravo and G. E. Galvez), and Cornell University (D. H. Wallace and R. E. Wilkinson). There may be other breeding programs in addition to these with which the author is unacquainted.

The Xanthomonas diseases of bean result from an intricate interaction of pathogen, host, and environment. Among the intricacies of this interaction are pathogenic variation, developmental stage of host, method and site of inoculation, and method of evaluating the disease reaction.

1 Pathogenic Variation

The presence of pathogenic variation in X. phaseoli was initially suggested in 1956 by Smale and Worley (11) who detected pathogenic differences in individual colonies of stock Xp cultures. A year later, Corey and Starr (3) isolated from a stock Xp isolate four colonial mutants which differed in pathogenicity, pathogenicity, in terms of lesion number and size was directly proportional to the amount of extracellular polysaccharide produced in liquid medium. Neither of

these papers actually reported comparative virulence of several different isolates, but only of sub-isolates within a stock culture

Definitive evidence that variation is present in Xp isolates from various geographical areas was presented by Schuster and co-workers in Nebraska (8,9,10) Xp isolations from seed of Colombian (isolates C6 and C7) and Ugandan (isolate U2) dry bean cultivars were more virulent on the 'tolerant' bean selection, Great Northern (GN) Nebr #1 sel 27 than was the standard Nebr Xp S isolate. The authors suggested that, in view of the high virulence of certain Xp isolates, pathogenic variation may be an important factor in the development of tolerant varieties.

Ekpo and Saettler (5,7) confirmed the existence of pathogenic variation in Xp and extended the existence of such variation to Xpf as well. One of the Michigan Xp isolates (Xp 15) which they tested was somewhat more virulent on GN Tara, Jules, and Nebr #1 sel 27 than were the C6 and C7 isolates mentioned previously. Xp isolate U-2 was also confirmed to be quite virulent on GN Nebr #1 sel 27.

In general, Ekpo and Saettler found that Xpf isolates were somewhat more virulent on the bean varieties tested than were Xp isolates. For example, Xpf isolates 16 and 19 from Michigan, 844 from Guatemala (via Nebr) and CIAT A from Colombia (via Nebr) incited severely susceptible disease reactions on GN Nebr #1 sel 27. Only 2 of 8 Xp isolates incited a similar reaction. In addition, although all eight of the Xp isolates incited a tolerant or slightly susceptible reaction in PI 207262, four of the Xpf isolates incited a moderately- or severely-susceptible reaction on this line. These authors also point out that extremes of pathogenic

variation in Xp and Xpf could well be greater than that reported

There is controversy regarding the taxonomic differentiation of Xp from Xpf on the basis that Xpf produces a brown, soluble pigment in certain culture media while Xp does not (1,2,4,9) This controversy, in turn, is associated with conflicting reports that Xpf is more virulent (6,7,12) or not more virulent (13) than Xp Brown pigment production is stable for Xpf and can be used to separate it from Xp (1) However, separation of such closely-related pathogens as Xp and Xpf on this basis is unnecessary, and separation on the basis of pathogenic variation is invalid What is important, then, is to realize that there is extreme variation in pathogenic potential of these organisms, and that understanding this variation is crucial to the development of widely-adapted Xanthomonas tolerant bean cultivars

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2. Developmental Stage of Host

That bean leaves of different ages are not equally susceptible to Xp infection has been known for some time Goss reported that as leaf age increased, Xp susceptibility also increased (17) On the other hand, Patel and Walker noted that the youngest rather than the oldest leaves were the most susceptible (19) Both of these studies were made with plants in the vegetative stage of development, however, and did not simulate a natural field situation of Xp disease development

In the field, the Xanthomonas diseases become most visible at or just following the blossom stage, generally, symptoms are observed initially on the lower, older leaves Secondary spread of the pathogen occurs most rapidly after this time The importance of evaluating breeding material in various stages of development was emphasized by Coyne et al who determined that plants of both susceptible and 'tolerant' lines were more susceptible to Xp when in a reproductive stage of development (14,15, 16). Increased susceptibility to Xp and Xpf when plants are in reproductive as compared to vegetative stage of development was recently reported to be a common phenomenon by Saettler and Ekpo (7) There was a strong suggestion of higher virulence of Xpf isolates to reproductive rather than to vegetative plants as compared to the Xp isolates In some isolate/host combinations, however, the opposite phenomenon resulted whereby vegetative plants were more susceptible to Xp or Xpf than were reproductive plants

Even though developmental stage is important when evaluating breeding material for Xanthomonas tolerance, previous work on inheritance of Xp tolerance based on inoculation of vegetative plants is not negated (18,20) This is especially true for Honma's successful crossing of P. acutifolius var. latifolius (Tepary bean) with P. vulgaris cv Great Northern, and identifying at least some of the progeny as highly Xp-tolerant GN Nebr. #1 resulted from this interspecific cross, and sel 27 was in turn selected out of GN Nebr. #1 (16) This sel 27 has been used extensively as a source of Xp and Xpf tolerance in other breeding programs

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3 Method and Site of Inoculation

Numerous methods of inoculation have been employed to screen for Xanthomonas tolerance in beans Perhaps the two most common methods are water-soaking (22,23,29) and single-or multiple-needle pricking (21) of leaves Ekpo (5) compared multiple needle, and leaf water-soaking inoculation methods using one isolate each of Xp and Xpf on bean cultivars Tara and Mich-Cal Cranberry Extremely variable levels of necrosis and chlorosis developed with the needle inoculations, whereas very reproducible leaf lesion symptoms developed with water-soaking method One can somewhat question the needle inoculation technique also on the basis that it causes a fair amount of artificial tissue injury Arp, et al (22) have also noticed that, under field conditions, the leaf water-soaking method is extremely useful in showing differences between susceptible and tolerant cultivars

Ekpo described a leaf-incision technique for primary and trifoliolate leaf inoculation which was useful in 'typing' pathogenic variation, however, there was no useful correlation of leaf-incision symptoms with the field reactions of both susceptible and tolerant cultivars

In addition to method of inoculation, the quality and quantity of inoculum are important in detecting Xanthomonas tolerance in beans. That quality of inoculum is important has already been discussed under pathogenic variation, this author believes that breeding material should be exposed to a wide, geographical spectrum of pathogenic potential. Quantity of inoculum is important in that field-tolerance may be overlooked if too high of an inoculum concentration is used.

Coyne, et al (16) alluded to the importance of inoculum concentration in screening for reaction to Xp (they employed the multiple-needle inoculation method). Ekpo (5) studied the relationship between inoculum concentration and disease reaction in susceptible Manitou bean cultivar using 8 Xp and 7 Xpf isolates under greenhouse conditions, the water-soaking method of inoculation was used on 18-day-old-plants. Disease reactions of plants under these conditions correlated with reactions in the field only when an inoculum concentration of 2.8×10^7 cells/ml was used, 2.8×10^3 and 2.8×10^5 cells/ml generally gave disease reactions of tolerant or slightly-susceptible, Coyne et al found 5.41×10^7 cells/ml optimal in their study (16).

In the absence of useful selective media for Xp and Xpf, it is impossible to determine inoculum concentrations of these pathogens in the field. It would be extremely useful to monitor actual Xp and Xpf cell concentrations during secondary disease spread in the field, this would allow one to more carefully inoculate bean lines with an inoculum concentration as it occurs in the field.

Most of the Xanthomonas breeding programs rely upon field inoculations using the water-soaking technique to identify tolerant materials. Breeding lines are usually inoculated directly with the bacterial suspension just at or after the blossom stage. In the Michigan-USDA program, however, only alternate 'spreader' rows are inoculated, and evaluations are based on the degree of secondary disease spread into the tolerant lines (28). Both of these procedures are useful and result in quite reproducible blight ratings.

Pod inoculation with blight bacteria is useful for detecting virulence (26,30) and could be a useful technique for identifying tolerant lines (5,24). Hill et al (25) have shown that pod reaction to the halo blight pathogen (Pseudomonas phaseolicola) is under genetic control, this may also be the case with Xp and Xpf. Tolerance to leaf infection does not insure tolerance to pod infection with Xp and Xpf, and Coyne and Schuster suggest combining high foliage with high pod tolerance (24). Ekpo (5) has confirmed the suggestion of Coyne and Schuster in that pods and foliage of both tolerant and susceptible bean cultivars may react independently in response to Xp and Xpf inoculation.

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4 Method of Evaluating Disease Reaction

Most of the rating scales used to define disease reactions of inoculated plants are quantitative, as well as qualitative in nature. For example, Coyne and Schuster (8,10,16,22) generally use the following scale

- 1 T = Tolerant, no visible symptoms
- 2 SI-S = Slightly susceptible, small lesions develop on a few leaves
- 3 Mod-S = Moderately susceptible, moderate leaf lesions develop with some chlorotic lesions
- 4 Se-S = Severely susceptible, many large lesions develop on most of the leaves with pronounced necrosis and chlorosis
- 5 Very severe infection, plants chlorotic, necrotic and largely defoliated

Saettler and Adams (28) have used the following scale in evaluating breeding lines for Xp and Xpf tolerance

<u>Rating</u>	<u>Symptom Type</u>
0 0	no disease
1 0	a few blight lesions, 5% leaf infection
2 0	5-10% leaf infection in the row
3 0	10-20% leaf infection, lesions large and spreading
4 0	25-50% leaf infection, many lesions coalescing
5 0	50-100% leaf infection, most plants dead

Lines developing a disease rating of 0 0-1 8 are generally classified as tolerant, and >1 8 are classified as susceptible. The actual 'cut-off' value between tolerant and susceptible varies from test to test, and is determined by comparing the disease ratings of numerous susceptible and tolerant checks in the Nursery.

A standardized blight rating scale should be developed and utilized uniformly by those involved in screening for Xp and Xpf tolerance in the field. At the same time, methods of inoculation should be standardized as much as possible so that a particular blight rating will mean the same thing to breeders and pathologists alike around the world.

One possible complication relative to blight rating scales is that absence of visual disease symptoms in inoculated tissues (or tissues infected from secondary spread) does not guarantee absence of Xp or Xpf. Indeed, several workers have shown that symptomless tissue of tolerant (5,16,31,32) or susceptible (5,33) bean cultivars can contain detectable levels of Xp or Xpf. It is suggested that additional research studies be initiated on the epi- and endo-phytic phases of Xp and Xpf relative to tolerant and susceptible cultivars. Tissue from field-tolerant lines should periodically be assayed to guarantee absence of Xanthomonas pathogens.

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5 A Cooperative Approach for Screening Phaseolus vulgaris L. germplasm for Xanthomonas tolerance

A Initial Assumptions

- 1 Standardized method of inoculum preparation, including quantity (concentration) and quality (pathotypes to be used)

- 2 Standardized method and time of inoculation using the water-soaking technique (direct inoculation of test plants, or use of 'spreader rows' for secondary spread)
- 3 Inclusion of at least two susceptible and two tolerant check varieties
- 4 Standardized method of evaluating disease reactions

B. Proposed Procedures

- 1 Xanthomonas Blight Nurseries would be conducted in the field by interested programs using the above assumptions. In the interest of phytosanitary considerations, it is suggested that initially, only indigenous Xp and Xpf isolates be used for artificial inoculations in the field
- 2 Continuous, and expanded efforts, should be made by each program to identify new sources of blight tolerance. Since transgressive segregation is now known, a much broader genetic base for tolerance is feasible, and attainable
- 3 Since most programs have, as an end product, a blight-tolerant cultivar with rather specific agronomic traits, crossing and selection procedures will be rather individualized. The inheritance of resistance in new sources of tolerance should be ascertained, however, if at all possible
- 4 Free exchange of tolerant lines, bacterial cultures (with appropriate care in their use), and expertise is not only encouraged, but also demanded if we are all to succeed in developing Xanthomonas tolerant bean cultivars or lines for our individual programs

(Bean Protection Workshop, CIAT, December 1-3, 1975)

1 SURVIVAL OF PLANT PARASITIC BACTERIA OF TROPICAL PLANTS

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4 U S A

5 Although my specific assignment is on survival of plant parasitic
6 bacteria of beans grown in the Tropics, it behooves me to expand the
7 topic to other bacteria and crops in the Tropics, and also include
8 some work done in the temperate zone, in anticipation that the infor-
9 mation can be applicable and extrapolated to bean bacteria

10 But before we get into this topic, let me offer some
11 introductory statements

12 The plant parasitic bacteria and other microorganisms that exist
13 today have become adapted to survive under current conditions. Sur-
14 vival of the species is the important terminus in organic evolution.
15 For plant pathogenic bacteria, it is the most immediate aspect of
16 survival that concerns us. Not many natural abodes or habitats offer
17 microorganisms any great degree of continuity of environments or
18 conditions, and an important hurdle for the species is the bridging
19 of interruptions in its environment, this includes discontinuities
20 in the supply of plant hosts--

21 With crop plants and crops artificial culture is uninterrupted,
22 or nearly so, either spatially or in time, and the presently survival
23 problem for the parasite is thereby taken care of. (Such an example
24 may occur in the tropics with perennial crops or with a succession
25 of crops the dry and wet periods in the tropics would provide a dis-
continuity of crops, however, because both periods are too favorable
necessarily)

26 Agricultural or horticultural methods arrange some modicum of
27 discontinuity between related host crops. This interruption may,
28 nevertheless, be less important than that which exists in nature,
29 and, therefore, the properties evolved by the pathogens for bridging
30 natural gaps are more often sufficient for bridging those in arti-
31 ficial plant culture. Problems of disease control are in direct
32 proportion to the ease with which a pathogen can transfer from one
33 crop to another, either in space or time. Uniformity of crop germ-
34 plasm favors inoculum buildup and perhaps perpetuation of the patho-
35 gens. In a recent report on "Genetic vulnerability of Major Crops,"
36 it is reported that bean in U S A have a narrow germplasm base
37 (Two types, the Michigan navy bean and the Pinto account for 60% of
38 all dry beans grown in U S A. Two pinto vars --Pinto 111 and 114--
39 account for almost all the acreage of pintos. All four navy bean
40 vars derive over 90% of their germplasm from the variety Michelite

1 It is clear that much of the edible dry bean acreage in U S A rests
2 upon a dangerously small germplasm base This is true for the navy
3 bean The situation is less critical for the Pinto class because
4 the production is dispersed in several different states, whereas the
5 navy beans are produced in a single concentrated region in Michigan
6 Most of the dry beans are susceptible to common and fuscous
7 bacterial blights

8 To control diseases, one has three courses to follow

- 9 (1) One may seek means to reduce the severity of disease,
10 once a plant or crop is infected
- 11 (2) One may seek methods or ways to prevent infection, once
12 the pathogen has reached the surface of the host plant, or
- 13 (3) One may seek to prevent any contact between pathogen or
14 plant host

15 There are several explanations why the third approach is the
16 most desirable, and it can be accomplished by interfering with the
17 normal mechanisms of the pathogen for overcoming its environmental
18 discontinuities The transfer mechanisms that pathogens have
19 adopted are related to the types of discontinuities that they face

20 These fall into two categories--spatial and temporal (time)
21 Spatial transfer is from one crop to another distant but contem-
22 porary crop Fungus and viral pathogens are notably successful at
23 this type of dissemination However, in these two groups some
24 important pathogens have solved their problems by temporal rather
25 than spatial spread Nematodes are of limited mobility at best, and
26 host finding is normally by persistence in soil

27 Plant-pathogenic bacteria, as opposed to animal pathogens, are
28 also mainly without any special adaptation to spatial dispersal,
29 although there are important exceptions

30 A sufficient amount of inoculum must be able to survive the
31 off-seasons in order to re-establish the pathogen when favorable
32 conditions are again presented It would be presumed that obligate
33 parasites (those that require a living host to grow) are more handi-
34 capped by discontinuous growth The main effect of growth stoppage
35 of the pathogenic bacteria is the decrease in the amount of potential
36 inoculum (The term inoculum refers to bacteria that, when placed
37 in suitable contact with the host plant, cause disease)

38 The relative success or failure of a plant pathogenic bacterium
39 depends on the amount of inoculum In the case of bacterial plant
40 diseases, the bacterial cells themselves are the inoculum Pathogens,

1 such as bacteria, which have a short disease cycle, can be expected
2 to develop more rapidly toward epidemic proportions because of the
3 rapid inoculum buildup from a small amount of surviving primary
4 inoculum

5 A point at this time concerns the minimum amount of inoculum
6 necessary to initiate disease. Large amounts of primary inoculum
7 are of little or no value if successful transmission to a susceptible
8 host plant does not take place. Therefore, it is suggested that the
9 source, exit, and transmission of the primary inoculum into new crops
10 should also be considered. This troika is required for disease to
11 occur, for survival, and for continuity of the bacterial species.
12 Knowledge of these factors could be important in control by estab-
13 lishment of a "curtain" between host and the primary inoculum phase
14 of the pathogenic bacterium. Control might be a simpler and cheaper
15 task in the primary inoculum phase than secondary inoculum phase.
16 The population levels are usually at their lowest point during off-
17 seasons and more susceptible (and amenable) to control. Plant para-
18 sitic bacteria dissemination is a difficult task to prevent, once the
19 disease is established. (The population levels are very high at this
20 stage.)

21 Two extreme dispersal patterns can usefully be characterized by
22 their efficiencies, ranges, and results. 'Imperative dispersal,'
23 which must be repeated for the organism to survive, is usually the
24 means whereby the bacterium exploits its habitat. It is typically
25 thorough, often frequent and specialized, and may employ many agen-
cies, including the growth of the host. There are many intermediates
between this and the other extreme of 'capricious (changeable, fickle
dispersal,' which is occasional and, although it may spread an organ-
ism, is not necessary for it to survive. Because it must operate at
greater ranges than imperative dispersal and need not succeed, it is
less frequent and can use less efficient methods. But the area,
time, and number of bacteria are so enormous that eventually it will
be successful--this is the game of chance, on the other hand, the
imperative dispersal is a game of skill.

19 The longevity of the primary inoculum is an important feature in
20 the success of bacterial pathogens and depends upon its ability to
21 escape or endure adverse environmental conditions. Survival may
22 depend upon external (physical and biological environment) factors
23 as well as the internal make-up and form of the primary inoculum of
24 the pathogen. The combination of these factors affects the minimum
25 concentration of primary inoculum necessary for initiation of a
disease upon re-establishment of favorable infection conditions.

24 These are some of the points that need to be considered in sur-
25 vival mechanisms of plant pathogenic bacteria. Since there are about
200 different "species" variations in modes of survival are destined

1 to occur Different species illustrate different types and establish
2 basic concepts The bacterial phytopathogens occur in four families
3 () in two orders (),
4 and are represented by only 5 or 6 genera Pseudomonas (90 spp),
5 Xanthomonas (120 spp), Erwinia (and Pectobacterium) (17 spp),
6 Corynebacterium (12 spp) and Agrobacterium (7 spp)

7 Plant pathogenic bacteria are aerobic non-spore forming rods
8 All except Corynebacterium are Gram-negative, most are motile with
9 either polar or peritrichous flagella, but a few are non-motile
10 (atrichous)

11 Some bacterial diseases are locally or generally of great economic
12 importance For example, Pseudomonas phaseolicola (halo blight
13 of bean), Ps syringe (brown spot of beans and many other crops),
14 Xanthomonas phaseoli (common blight of beans), S p fuscans (fuscous
15 blight of beans) X vignicola (blight and canker of beans and cowpeas),
16 Corynebacterium flaccumfaciens (bacterial wilt of beans), Ps
17 solanacerum bacterial wilt of 200 species of musaceous and solanaceous
18 plants, including the bean, P savastoni (olive knot)

19 Wellman (1972) lists many bacterial diseases found in the Tropics
20 which are caused by Pseudomonas and Xanthomonas species In fact,
21 the first bacterial disease in the world was described in the tropics
22 (1869) about 20 years before Burrill sugarcane gumming caused by
23 X. vasculorum Wellman (1972) refers to 20 bacterial pathogens (in
24 6 genera) in Tropical America (Neotropics) that are fairly common
25 causes of plant diseases Presumably, these disease syndromes could
differentially affect the survivalability of the pathogens, directly
or indirectly

16 In bacterial disease, primary inoculum sources are the various
17 survival mechanisms adopted by the pathogens during dormancy imposed
18 by the growth periodicity of the host plant, or unfavorable periods
19 for infection and development winter in the temperate zones or the
20 dry periods in the tropics

21 Just as studies of dispersal are important in the control of
22 diseases, so, if duration of survival of pathogens is to be reduced,
23 it is important to understand the mechanisms of survival The
24 difficulties may, of course, be greater in some survival sites than
25 others

22 Mechanisms of survivalability can be presented by use of different
23 approaches Plant parasitic bacteria do not form resting
24 spores or structures comparable to other pathogens (nematodes or
25 fungi) and remain dormant during the quiescent period in association
with animate or inanimate agencies, such as

- 1 1 seeds
- 2 2 plant residues
- 3 3 perennial plant hosts or parts thereof
- 4 4 insects
- 5 5 epiphytes
- 6 6 soil and other non-host materials

Pathogen longevity will be referred to under natural and artificial environmental conditions

Pathologists have devoted much time and effort to the growth stage of the life cycle. It is this period that effects of bacterial growth are most noticeable: leaf spots, blights, wilts, galls and other symptoms that result in plant losses. Knowledge of the life cycles and survival may provide improved control measures. Presumably, the vulnerable period for bacteria is commonly during low population levels. One should attack the pathogen during this period. This period is usually between seasons or wet and dry periods (in the tropics). Reference is made here to Primary Inoculum. Primary inoculum survival is from season to season. Secondary inoculum survival refers to survival for minutes-days (short term).

The life cycle of a plant pathogenic bacterium can be divided into three phases: pathogenic, saprophytic, and survival phases. Leben has added a fourth, "resident," to include all types of associations of microflora with healthy plants. The pathogenic, saprophytic, and resident phases are considered as growth phases in the secondary infection category; the fourth phase could be considered as primary infection or inoculum. How is each phase involved in the production of surviving cells? It is a practical exercise to discuss how phytopathogenic bacteria survive during unfavorable periods. More and more we are trying to refine our comprehension of life cycles and modes of survival.

SHORT TERM SURVIVAL

Pathologists are mostly concerned with the practical effects of long-term survival. It might not be inappropriate to mention survival of metabolically and physically active bacteria for shorter periods - hours and days. Physical, chemical, and microbiological factors affect both short- and long-term survival, information on the latter is sketchy. Free water is required for bacterial reproduction and locomotion. Actively growing asporogenous bacteria existing independently succumb quickly when exposed to desiccation. Leben found that Pseudomonas glycinea bacteria on glass slides died within minutes after water suspensions were dried. High relative humidity favors epiphytic growth in general (Leben).

Plant parasitic bacteria on aerial plant parts probably are quickly decimated by ultraviolet rays of sunlight. UV irradiation kills bacteria (X p. var. sojense) on the leaf surfaces but not in the intercellular leaf spaces (Barnes, 1965). The effect of UV is influenced by relative humidity.

After infection and during disease development, pathogenic bacteria are subject to effects of other microorganisms. As the disease develops nonpathogenic microorganisms initiate decay of the lesions and of pathogens therein, this decay is more rapid when infection is in proximity to the soil. Survival time would depend on the rate of decay of the plant tissues. Interactions between bacterial species can influence population buildup and consequently survival. Prior inoculation with avirulent mutants may protect against virulent pathogens (Goodman).

Thus survivability of active cells is apt to be of short duration. Bacteria washed from an active lesion on foliage probably would die quickly due to desiccation and UV irradiation. This relationship should apply to epiphytic bacteria but this would depend on species and drying conditions. Pathogenic bacteria migrating from active lesions on leaf or root become inactive in the soil, by and large because of lack of energy sources and unfavorable conditions. Should energy source become available other organisms would be better able to use it, because plant pathogens are not nutritionally versatile and less active biochemically (Misaghi and Grogan, Sands and Schroth). Thus it seems likely that unless a suitable niche could be found quickly, migrating bacteria to soil would expire in a short time. There are a few exceptions and these will be mentioned later.

At this point, it might be stated that with respect to the subject of survival of plant pathogenic bacteria, surprisingly little work has been done on this part of the life cycle.

Three frameworks of fact and speculation concerning survival can be considered.

1 Maintained on the soil surface compared to incorporation 8" in soil
2 This indicates that bacteria survive in dry plant parts Companion
3 studies were run in the greenhouse in which these bean and soybean bac-
4 terial pathogens in infested tissues survived in dried soil (0 95% WHC)
5 and soon died in moist soil (30%WHC), these were maintained at tempera-
6 tures ranging from 80-120°F (25-35°C) This work at Nebr , U S A was
7 confirmed for X malvacearum (cotton blight) in Sudan (Africa) and Tang-
anyika to some degree In arid climate of Sudan infested debris is a
threat to the next cotton crop In Tanganyika where the rainfall is
considerably higher than in the Sudan, X malvacearum barely survived
between cotton crops on the soil surface Bacterial spot of tomato
(X vesicatoria) which overwintered in tomato and debris in Nebr , U S A
was introduced in transplants grown in southern U S A

8 X oryzae can easily survive in dried rice straw but survives only
9 1-2 months when the rice straw is plowed in the soil In the Philippines,
10 X oryzae was not recovered from rice stubble 18 days after the "Kresak"
11 stage when maintained in pots after removal of all but the basal 3cm
12 (Nwigwe, 1973), presumably the stubble was moistened which favored its
breakdown The warm humid climate of the tropics and the current trend
for continuous rice cropping favor the perpetuation of X oryzae, the
bacterial blight, as most of existing varieties are susceptible

13 The bacteria survive in dried lesions on infested tissues, if
14 moistened as would occur if debris is "plowed under" and exposed to
15 microbial decomposition, it is expected that surviving cells surrounded
16 by diseased tissue would not be so readily decayed as when imbedded in
17 smaller amounts Cells in woody tissues would be less subject to decom-
18 position than those in soft tissues This was well illustrated with LEW
19 disease of maize (C nebraskense) (slide) bacteria survived 10 months
20 in stem and crown tissue at 4 and 8" depths compared to their disappearance
in leaves and other softer corn tissues Debris from diseased plants
should be considered a possible source of carryover But how likely is
it that debris will decompose between susceptible crops If it is not
decomposed what is the likelihood that surviving bacterial parasites
will come in contact with susceptible tissues With our ordinary crop
cultivation methods, survivors in diseased leaves, etc , would occur
near susceptible seedlings rarely, if ever, particularly if crops were
rotated

21 SEFD

22 On the other hand, diseased debris associated with seed would be
23 kept dry and thus not subject to deterioration In this condition,
24 bacterial cells may well survive as long as the seed is viable Seed
contamination might occur during harvesting and processing, halo blight
25 was found viable in seed infested debris after 13 months (Grogan and
Kimble, 1967)

1 "The flowers of all the tomorrows are in the seeds of today"(Cowen,
2 1974) As vehicles to spread new life, seeds can be enemies to the sur-
3 vival of man when they carry unwanted parasites

4 Seed is considered the ideal agency in which plant pathogens sur-
5 vive periods when the host is lacking Orton (1931) indicated that any
6 of the bacterial pathogens is likely to be transmitted by seed. His pre-
7 diction is becoming a reality as the roster becomes greater

8 Bacterial pathogens that infect Phaseolus vulgaris and other le-
9 gumes serve as excellent examples of seed-borne pathogens Xp, Xpf, Xv,
10 Ps phaseolicola, P syr, C f, Cfa, Cfv Ps solanaceum which is
11 common in the tropics also can infect beans and may be seed borne, it
12 has been found in seed of soybeans and peanuts

13 The growth and development of the seedbean industry in the semi-
14 arid West of U S A was based on the relative freedom from several bac-
15 terial pathogens (Pp, Xp, Xpf, Cf and Psyr) But the bean industry sus-
16 tained disastrous economic losses as a result of severe outbreaks of
17 bacterial diseases 1963-1967 Reasons for this sudden appearance of
18 bacterial diseases were (1) occurrence of favorable weather, (2) intro-
19 duction of disease on seeds grown for increase, (3) and occurrence of
20 new races viz Race 2 of halo blight By a concerted effort by several
21 agencies the seed bean industry has cleaned up their bacterial problems

22 A few seeds infected can result in disastrous epidemics In Wis-
23 consin, a dozen seeds infected with Ps phaseolicola distributed at
24 random could result in an epidemic In Canada it was shown that 0.5%
25 seed infected with fuscous blight can be disastrous

26 The use of bacterial free-bean seed is a classic example of disease
27 control However the recent (1960's) outbreaks of bacterial diseases
28 in U S A. demonstrated how widespread epidemics can occur when seed is
29 produced in a localized area

30 In New South Wales (Australia) seedbean production is located in
31 high rainfall climates (compared to low rainfall areas in U S A)
32 Purpose is to form development of bacterial blights and anthracnose so
33 that trace infections of these diseases are readily detected In late
34 1950's and early 1960's acreages for certification in New South Wales
35 and Victoria declined because of the increasing costs and the consider-
36 able risk of wet weather at harvest (Ballantyne, 1974)

37 The drier climate of North Queensland (Australia) was less favor-
38 able for production of high germinating seed of some bean varieties
39 (stringless) than the moister climates of New South Wales In 1967,
40 Queensland Department of Primary Industries also required that equip-
41 ment used for planting, harvesting, and cleaning bean seed be cleaned
42 and disinfected with either a mixture of cetrimide and chlorhexidine

(Johnson and Arvier, 1967) or methyl bromide to minimize risk of contamination by bacterial blights in the Certification and Approval Schemes. (We checked MBr and found it does not affect germination of RK nor affect rubber (tires).

There is a need for decentralization of bean seed certification. From the experience in the 1960's concentration of production in Idaho, for example, is dangerous and creates a potentially vulnerable situation for users of this seed (Zaumeyer, 1972, Natl Acad Sci)

Similarly in Australia seed production of green and wax beans is concentrated in Northern Queensland (dry tropics) and unfavorable seasonal conditions and outbreaks of diseases have caused reductions in quality and quantity of bean seed produced some seasons. The location and development of other bean seed production areas in Australia is also desirable (Ballantyne, 1974)

Survival per se of a bacterial pathogen in seed or other agency is not the complete answer. Two seed-borne pathogens of corn (*E. Stewartii* and *C. nebraskense*) have negligible transmission to seedlings. The bean seed bacteria on the other hand are notorious for transmission, in greenhouse tests we find that halo blight is especially notorious in this respect.

Obviously bacterial diseases are also a problem in the tropics. We in Nebraska isolated from bean seed strains (C-6, U2) of common blight more virulent than those in Nebraska. Ex Xp C-6, C-5 from Colombia and Xp U-2 from Uganda (Africa). From Brazil, Colombia, El Salvador, Guatemala, Honduras we isolated Xpf and Xp. From bean seed from Colombia we isolated bacteria (Xv-C7) that attacks cowpeas (*Vigna unguiculata*) inciting stem canker and blight (MS and DPC 1975). This is comparable to X vignicola from sub-tropical Texas (U S A) (Burkholder, 1944), and Puerto Rico (Valili, et al 1975). This indicates dangers of seed exchange without proper precautions. Seed increases of such seed perhaps should be done in the greenhouse on individual plant basis.

Plant parasitic bacteria may reside in non-host materials, such as soil. It behooves us to differentiate between survival in infested plant residues and in soil. A case in point is the assumption that phascoli survived in the "soil" with the exact inoculum source not ascertained. Since then we have found that the pure cultures of bean bacteria do not survive in or on the soil, as such.

Plant parasitic bacteria can be categorized into three groups based on survival in soil (Ludden, 1965)

Most plant parasitic bacteria belong to the group that soil phase is a rapidly decreasing one. I mentioned the bean bacteria as belonging in this category. Citrus canker (X citri) of oranges etc., is another example in Florida, U S A. Japanese claim otherwise. But the fact that citrus canker was eradicated in citrus areas of the U S A by systematic destruction of diseased grove and nursery trees and sanitation would indicate that X citri is poor soil organisms. Citrus canker occurs sporadically in Tropical America but compared to Ps solanacearum is of little importance.

Ps solanacearum, which attacks about 200 plant species including beans, is probably indigenous to the tropics (Wellman P177, 1972). According to Wellman (1972) once Ps solanacearum becomes established in a region it becomes a permanent resident in the soil. Wellman and let me quote, wrote "This I have found is also true of so-called bacterial blight (Xanthomonas phaseoli) on bean and the sorghum bacterial stripe (Pseudomonas andropogoni). I do not have the references on bean blight but it is an interesting phase to investigate survival in the soil." End of quote.

As mentioned before, one must differentiate between soil and infested debris in or on the soil. Ps solanacearum and crown gall (Agrobacterium tumefaciens) may owe their long term occurrence in the soil because they may be host dependent with their populations in the soil increasing or gradually decreasing according to cropping practices. Nevertheless, these 2 bacterial species are the only ones that approach the true soil-borne category.

A common explanation for poor survivalability of plant pathogenic bacteria in soil is inhibition of antagonistic microflora, many actinomyces, bacteria, fungi are antagonistic for plant pathogenic bacteria. Bacteriophage unlikely affect bacterial ecology. Anderson () reported that under favorable in vitro conditions the lowest initial concentration of a virulent phage required to eliminate a single cell was in the vicinity of 10^7 particles/ml. Crosse (1948) found that plant pathogen bacteria (P syringae) have rarely exceeded 10^2 phage particles/ml of soil suspension. Since this yield is equivalent to the lysis of only 2 or 3 bacterial cells it is clear that the initial concentration of phage is very low and chance of absorption onto cells occurring, very remote. Sutton and Wallen

() found similar comparisons for X phaseoli of bean

EPIPHYTES

Evidence is accumulating that plant organs sustain a characteristic epiphyte bacterial flora. These have been found on roots, buds, and leaves. An interesting report was that of Hagedorn, et al (1972) who recovered the bean brown spot bacterium (Ps syringie) throughout the year on leaf surfaces of healthy vetch (Vicia villusa), and associated natural outbreaks of brown spot with these weed epiphytes. Haas () found under artificial inoculations X p var fuscans survived on surfaces of bean primary leaves but disappeared quickly from unifoliate leaves. Other workers have found similar relations with bacteria on other crops, soybeans, apple, cherry, citrus, pears.

Certain plant pathogen bacteria survive on root surfaces such as P tobaci and P angulatu on roots of wheat, vetch and weeds. P phaseolicala and X p var sojenise were found not to overwinter on wheat roots. Stanch and Lasik found that X p fuscans colonizing bean roots up to 2 weeks and then disappeared. These are a few examples of epiphytes surviving on surfaces of plant parts. We have found that X p and Cfa overwinter inside weeds, pigweed and goosefoot and could be involved in bean blight and wilt epidemiology. Weeds have been shown to be involved in many other plant diseases.

INSECTS

A few plant pathogen bacteria have adopted a specialized means of survival and persist in a characteristic manner. A classic example is Stewart's wilt that survives in the corn flea beetle. They were able to forecast the prevalence of this disease and weather conditions. The cucurbit wilt bacterium, E tracheiphila, is completely dependent on striped and 12 spotted cucumber beetles (Acalymma vittatum) for its survival between seasons. Other examples of insect survival and dispersal of plant pathogen bacteria are olive knot, potato blackleg, and certain diseases incited by Mycoplasmas, rickettsia-like bacteria (Pierces' disease of grapes, almond scorch and leafhoppers). Perennial plants can act as woody protected places for bacteria, fire blight of Posaceae, X juglandis (Pollen) P mori prunum, A citri, X pruni, holdover cankers or blighted twigs.

Two alfalfa pathogens, X alfalfa and C insidiosum can remain in the perennial crop from season to season.

1 Bacterial ring rot of potato (C s) also is perpetuated
2 in tubers and there are many other such diseases, orna-
3 mental, flowers (calla lily, hyacinth, nursery stocks,
etc) Preseume many tropical diseases are perpetuated
in perennial plants or parts

4 I would like to back-track for a moment concerning
5 bacteria and soil as a site of survival In Wellman's
6 book on "Tropical American Plant Diseases" are mentioned
7 certain points that may have a bearing on soil survival,
8 "Crude observations give evidence that soil bacteria in
the moist, warm tropics are very much more numerous than
in the cool, dry parts of the temperate zone The organ-
ic matter, though produced in great quantities, is
decayed at a high rate"

9 Another evidence of great bacterial effects on tropi-
10 cal soils is the occurrence of widely encountered
11 laterite formations These soils are red and not easily
12 cultivated for profit (large amounts Al_3 and Fe_2)
13 These are left from effects of intensive bacterial
action along with rains and warmth Laterite must be
disturbed as little as possible in cultural processes,
cover crops and mulches This if like minimum tillage
could affect survival in residues on soil surfaces

14 The bacterial content of tropical soils can be of
15 use in plant pathology There is speculation that bac-
16 terial populations have types that depend on fungus
17 hyphae in soil for existence Perhaps these bacteria
18 might affect plant pathogen bacteria Or possibly, the
tropical soils might favor tremendous buildup of plant
parasitic bacteria? Wellman did state that once common
blight of bean, (and brown rot (Ps solanacearum)), for
example, berome established it becomes a permanent resi-
dent of the soil

19
20 Bean halo blight, a serious disease, is probably
21 in all bean growing areas of the New Tropics (Tropical
American) except, perhaps where it is very dry

22 Bean fields of many countries in Tropical America
23 are severely attacked by common blight (X p)
24 The disease often becomes intensif ed in severity in a
25 few years In El Salvador, starting with a few plants
in a field affected, in 3 years time the crop was
practically destroyed by this blight Attempts to
change the cultural practices of the farmers were not
successful The farmers insisted on keeping the kind

1 of beans they have eaten for generations and any sort of
2 crop disease control was resisted. The village had to
3 move to another side of the volcanic slope. Similar
4 problems had been present in the U S A. But disease-
5 free seed acceptance will be a problem in case resistance
6 is strong. Field demonstrations do help in "educating"
7 the growers, this is based on personal experience

8 CONCLUSION

9 The thrust of these comments has been to emphasize
10 that plant pathogenic bacteria are poorly adapted for
11 survival in nature away from plant tissues. They
12 survive off-season in aggregates in association with
13 infected dry plant tissues or as individuals in protect-
14 ed places associated with healthy plant or plant part
15 (resident). Surviving cells are possibly in a dormant
16 or hypobiotic state rather than in an active condition
17 most often studied in the laboratory

18 In nature, the pathogenic phase of the life cycle
19 probably contributes most of the cells that carry the
20 bacteria through adverse periods. The contribution of
21 the resident phase (bacteria in or on healthy plants
22 or plant parts) may well be important, but the extent
23 of its natural occurrence is unknown. On the other
24 hand, the saprophytic phase seems to contribute few
25 cells that survive (?)

Now if these presentations have validity, it is
clear that we need to study hypobiosis, and the nature
of protected survival sites and how pathogenic bacteria
get into them. With more knowledge, we may learn how
to change hypobiosis and expose protected positions to
our advantage

19 CONTROL METHODS

20 Control of bacterial diseases of beans is essential
21 because they are epidemic type diseases. Adequate con-
22 trol can be accomplished in several ways. Eradication
23 of the pathogen, and development of genetically resis-
24 tant bean varieties are accepted measures. The latter
25 is a long range program, one that has potential and
is the ideal method but requires intensive efforts by
all concerned. The former can be accomplished, but
will require the cooperation of all the segments of the
seed bean industry

1 All students of plant pathology know there are three
2 factors to consider in a control program They are

3 1 The presence of a susceptible host

4 2 The presence of the pathogen

5 3 A means of moving the pathogen to the
6 susceptible host

7 If any of these three factors can be eliminated, control
8 can be successful

9 For example, if all bean plants are resistant or
10 immune to the pathogen, the problem is controlled,
11 P acutifolius has resistance to all strains tested
12 of Xp, Xpf and is resistant to one isolate of X
13 vigincala (C 7)

14 The problem is in crossing tepary with P vulgaris, as
15 requires embryo culture Perhaps by modifying the agar
16 medium better success may be realized

17 (2)

18 If the pathogen is eradicated from the area of
19 susceptible plants, control is accomplished, seed
20 certification (disease free seed) Check seed for
21 presence of bacteria by soaking 200 gm seed in buffered
22 solution and water soaking leaves with atomizer attached
23 to 15 psi Or test seed stocks by serology, bucket
24 testing, plant inoculation, UV light, Then foundation-
25 type program can be initiated Decentralization of seed
been areas is necessary, recall U S A in 1960's and
disaster

(3) If means of spread is eliminated, disease control
is possible Crop rotation, deep plowing (fall)
decrease primary inoculum However, in the Tropical
America, conditions in soil survival might be different
from much of the work done on beans elsewhere

1 I am grateful to you for this opportunity to appear
before you and for your patience

2 In closing, I might add an additional word of thanks
3 to Dr. Guillermo Galvez, the organizing committee, and
4 CIAT for the privilege they provided us to meet and
exchange ideas on the subject of bean bacterial diseases
5 that dominates several of our professional lives and
possibly even haunts us at times

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SEED-BORNE DISEASES OF SOYBEANS

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Introduction Plant pathologists and soybean breeders have become increasingly aware within the past two years that the single most important factor in the so-called "yield barrier" in soybean production is a pathological one. The organisms of greatest economic importance cause diseases of the roots, stems and seeds. Most of the important pathogens are seed-borne. Only within the last year has the effect of seed-borne microorganisms on seed quality, seedling vigor and yields been understood.

Microorganisms and viruses associated with soybean seeds There are about 100 genera of fungi, nine species of bacteria, about 30 genera of nematodes, and about 5 viruses reported associated with soybeans (14, 19, 20, 21). About 100 microorganisms are associated with the seeds (20, 21). In a recent study on the microflora associated with surface sterilized soybean seeds grown in Ethiopia, Mengistu (5) reported 20 genera of fungi as new records for the world.

The soybean seed coat is the tissue most frequently colonized. Histopathological studies have shown the following microorganisms colonize the "hour-glass" cell layer of the soybean seed coat, which is starch-rich (16, 18). Bacillus subtilis (S. R. For, UNIC, personal communication), Cercospora kikuchii (6), Colletotrichum atramentarium var. truncatum (16), Diaporthe phaseolorum var. sojae (6), and Peronospora manshurica (4). It appears that the dead organic tissue of the soybean seed coat can support many types of microorganisms.

Diaporthe phaseolorum var. sojae (Phomopsis sojae), causal agent of the pod and stem blight disease, was found internally seed-borne among six varieties from six states in the U.S., India, and Ethiopia (3, 5, 7, 8, 11, 15, 22). The percentage occurrence varied with harvest year, location and variety (3, 7, 8, 22). When the percentage occurrence approached 25 percent or more in any one seed lot, *in vitro* germination and field emergence were reduced (3, 7, 8, 11, 12, 22).

Besides the organisms listed above, other seed-borne fungi studies were Aspergillus flavus (1), A. mellis (2), and Macrophomina phaseolina (11). Pestalotia sp. and Thielavia basicola were reported for the first time to be seed-borne in U.S.-grown seeds (10). Species of Pythium, Botrytis, Cladosporium and Chaetophoma were reported for the first time to be internally seed-borne in seeds grown in India (17). Seed-borne fungi varied quantitatively and qualitatively among cultivars and locations (2, 3, 7), planting date (11) and storage conditions (11, 13).

During routine examination of seed-borne organisms in soybean at the University of Illinois, a bacterial growth commonly occurred over the surface of many seeds when surface-sterilized and plated on synthetic media (9, 11, 13, 22, 23). We have considered the bacterium to be P. glycinea, but now know it is Bacillus subtilis. Bacillus subtilis was detected in soybean seeds harvested in six states of the U.S.

and India (9, 13, 22). There were no significant differences in occurrence between states, but there were differences among varieties (21) and among different seed lots of the same variety (9, 11, 13, 22). The bacterium reduces germination, emergence, emergence and yield (9, 11, 13, 22, 23). The greatest reduction in emergence of seedlings from infected seeds was in soil at 15°C (23) and the greatest recovery of the bacterium *in vitro* was at 30°C (unpublished).

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CHEMICAL CONTROL OF SEED-BORNE PATHOGENS
IN SOYBEAN

by

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Introduction A few years ago this topic would be confined to the use of compounds in the form of dusts, wettable powders, slurry, or in-the-furrow treatments applied to seeds. The application of fungicides and antibiotics to soybean seeds is restricted if the seeds must also be inoculated with Rhizobium japonicum. Also, soybean seed coats readily imbibed water and the use of liquids or a slurry softens the seed coat so that planting is difficult. There have been some interesting developments in the chemical control of seed-borne pathogens in soybean.

Seed treatment prior to planting There are a number of studies on the uses of various seed-treatment compounds on soybean seeds (22). It has been considered that little benefit could be gained from treating soybean seeds. However, there has been a renewed interest in soybean seed treatment with the introduction of systemic fungicides (21) and restricted use of mercury. In general, poor quality seeds (70% germination or less at 25 C, low seedling vigor and 10% or more internally-borne fungi) benefit more from seed treatment than good quality seeds (above 70% germination, high seedling vigor and less than 10% internally-borne fungi) (4, 9, 14, 15, 17, 21, 22, 23).

Generally, the best seed treatment materials are those that contain thiram (4, 9, 14, 15, 17, 25). A favorable response using penicillin suggests that bacteria as well as fungi may be involved in the seed-borne disease complex (15, 16, 20).

A method was developed to introduce seed-treatment compounds into soybean seeds without etting them (2, 20). Dichloromethane (methylene chloride) (DCM), facilitated the movement of thiazabendazole (TBZ), methyl 2-benzimidazolecarbamate (MBC), and penicillin into dormant soybean seed. Seeds treated with MBC + DCM and TBZ + DCM had less internally-borne fungi and Diaporthe phaseolorum var sojae (Phomopsis sp.) (13) higher germination in vitro and emergence in vermiculite and soil than nontreated seeds or seeds treated with DCM alone. DCM appeared to have some antifungal properties.

Application of fungicides in the field One of the important factors affecting soybean seed quality is the amount of internally seed-borne microorganisms (1, 3, 4, 5, 6, 7, 10, 11, 18, 19, 24). Diaporthe phaseolorum var sojae (Phomopsis sp.) (13) is one of the most frequently found fungi associated with poor seed quality (1, 3, 5, 6, 9, 10, 11, 14, 15, 17, 19, 24). Seeds infected with this fungus rarely germinate. The occurrence of this fungus and other fungi increases when harvest is delayed (1, 8, 11, 19).

The foliar application of benomyl and other fungicides on soybean plants significantly reduces the amount of internally seed-borne *B. phascolarum* var *sojae* and other fungi (1, 5, 7, 8, 10, 11, 18, 19, 22) and partially controls the incidence of seed-borne fungi and decrease in germination associated with delayed harvest (1, 8, 11, 19). Some of the fungicides that have been found effective are benomyl, thiophanate-methyl, thifludazole, chlorothalonil, and mancozeb (1, 5, 7, 8, 10, 18, 19, 22).

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SOYBEAN SEED PATHOLOGY STATUS AND RESEARCH

by

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Introduction Plant pathologists and soybean breeders have become increasingly aware within the past two years that the single most important factor in the so-called "yield barrier" in soybean production is a pathological one. The organisms of greatest economic importance cause diseases of the roots, stems and seeds. Most of the important pathogens are seed-borne. Only within the last year has the effect of seed-borne microorganisms on seed quality, seedling vigor and yields been understood.

Microorganisms and viruses associated with soybean seeds There are about 100 genera of fungi, nine species of bacteria, about 30 genera of nematodes, and about 15 viruses reported associated with soybeans (13, 17, 18, 19). About 100 microorganisms are associated with the seeds (18). In a recent study on the microflora associated with surface sterilized soybean seeds grown in Ethiopia, Mengistu (11) reported 20 genera of fungi as new records for the world.

The soybean seed coat is the tissue most frequently colonized. Histopathological studies have shown the following microorganisms colonize the "hour-glass" cell layer of the soybean seed coat, which is starch-rich (16) -- Bacillus subtilis (S. R. Foor, UIUC, personal communication), Cercospora kikuchii (8), Colletotrichum dematium var truncatum (16), Diaporthe phaseolorum var sojae (8), and Peronospora manshurica (6). It appears that the dead organic tissue of the soybean seed coat can support many types of microorganisms.

Effect of seed-borne microorganisms on yield Illinois-grown seeds of the cultivars Amsoy, Buesson, Wayne, Williams, and Wells were planted in small field plots under irrigated conditions near Davis, California in 1974. Seeds from this planting and Illinois-grown seeds of the same cultivars were planted in a randomized, replicated field plot at the University of Illinois in 1975. There was some variation in plant height and plants harvested, but plants of all cultivars from seeds produced in California yielded better than those from Illinois-grown seeds. Bioassays showed that seed grown in California had a greatly reduced microflora.

Nicholson et al. (12) showed that there was a significant reduction in yield of soybean plants grown from seeds artificially inoculated with a suspension of Bacillus subtilis by vacuum infiltration.

Indirect evidence of the effect of seed-borne microorganisms on yield was presented by Prasanna et al. (14). They showed that the 500-seed weight from soybean plants sprayed with benomyl, thiophanate-methyl, chlorothalonil, thiazendazole or manco + zinc was significantly higher than that from nonsprayed plants. Ross (15) found that yields from benomyl-sprayed plant under irrigation were significantly higher than nonsprayed plants. Horn et al. (7) reported that

yields of three cultivars of soybean were significantly increased when sprayed with benomyl, triphenyltin hydroxide or thiabendazole and yields of two other cultivars were increased significantly when sprayed with benomyl. Yield increases were due to seed weight rather than seed number.

Effect on in vitro germination, field emergence and seedling vigor Soybean seeds inoculated with an isolate of Aspergillus flavus had a lower germination than noninoculated seeds (3). Aspergillus melleus reduced the germination of inoculated seeds and caused a seedling blight (4). Diaporthe phaseolorum var sojae (Phomopsis spp.) (10), causal agent of pod and stem blight (18, 19), was found to be internally seed-borne among all cultivars in the U.S. with the percentage occurrence varying with harvest year, geographical location, and cultivar (2, 5, 7, 18, 19, 20). Low rates of germination were associated with high percentages of D. phaseolorum var sojae-infected seeds (1, 2, 5, 7, 10, 11, 14, 15, 20, 22). When the occurrence of this fungus approaches 25 percent or more, in vitro germination and field emergence are reduced. Exudates from seeds can increase susceptibility to Pythium spp. (9). Low temperatures can predispose soybean seeds and seedlings to infection by Pythium spp. (21).

Bacillus subtilis can reduce in vitro germination, emergence and yields (personal communications, 20). High soil temperatures can predispose soybean seeds to decay by B. subtilis.

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IDENTIFICATION OF LEGUME VIRUSES IN THE FIELD BY SEROLOGY

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Natural infection of legumes by viruses is common throughout the world. Identification of the causal virus is accomplished by a number of techniques varying in sensitivity, specificity and/or reliability, as well as in the ease with which they can be performed. Unless field-collected tissue can be used directly for the identification tests, transmission of the virus to other hosts by means of rubbing extracted sap onto leaves or by the use of biological vectors is necessary. This step may eliminate one or more of a complex of viruses causing the disease. Testing field-grown plants for virus infection may also become important in indexing seed stocks for the presence of seedborne viruses.

Inoculation of virus to a number of differential or diagnostic host species and observation of the response may enable identification of the virus, but slight changes in pathogenicity may lead to the erroneous conclusion that a new virus or strain has been found. The use of this type of bioassay for routine indexing is effective, but large amounts of greenhouse space and labor are necessary.

Direct detection of virus infection is possible by electron microscopy, both by the leaf dip procedure and by thin sectioning, but identification can be made only if other techniques are applied to confirm the identity. Slight differences in diameter or rod length is no longer considered evidence that a new virus has been isolated.

Serological reactions between a plant pathogenic virus and its specific antibody, have proven very useful in determining relationships between viruses.

1970

and in virus identification (van Regenmortel, 1966, Matthews, Noordham, 1973).

Serology has also been extremely useful in the nomenclature and taxonomy of viruses, enabling the grouping of similar viruses and/or separation into "serotypes". Elimination of a number of different names for the same virus has been made possible by serology. Distant relationships may be demonstrated by using high-titered, relatively broad spectrum sera that react with a number of virus isolates. Precise identification of viruses, however, is best done with lower-titered, relatively specific sera. Serum prepared from intact virions is generally more specific than that made by injecting dissociated virus protein (Shepard et al., 1974) and may be preferred for diagnostic purposes. In all serological reactions, of course, precautions must be taken to avoid antibodies to host antigens that may interfere with interpretation of the reaction. Ideally, all host antigens are removed from the virus by purification prior to injection into the rabbit.

A number of techniques for performing serological tests with plant viruses have been recently summarized by Ball (1974). Some of the tests which can be performed successfully with field-grown tissue include 1) the Ouchterlony double diffusion test in agar, 2) the single or radial diffusion in agar, 3) flocculation or agglutination of a solid matrix onto which specific antibodies are adsorbed, 4) precipitation tests in a liquid medium, particularly if analyzed by sucrose density gradient centrifugation, and 5) observation in the electron microscope of antibody bound to virus particles in a leaf dip preparation. A test selected for field diagnosis should be rapid, simple and reliable, and should require a minimum of facilities, time and equipment.

Of the tests mentioned, the latter two require an ultracentrifuge or an electron microscope, and thus their use on a routine basis may be limited. Their specificity, however, is very high since in both cases the virus-antibody complex is observed directly. Most legume viruses attain a sufficiently high concentration in tissue to enable the detection of virus from clarified sap by photometric scanning of centrifuged sucrose density gradient columns. Electron microscopy is useful primarily with rod-shaped viruses. The latex flocculation tests have been used recently by Barnett and Gibson (1975) with clover yellow vein virus and other clover viruses. This test is very sensitive for detecting low concentrations of virus, but its specificity is subject to a number of factors, such as temperature, genotype of host, environment of host growth, antigen antibody ratio, serum variability, and the need for a high-titered serum (1:2000 or above). Thus, although the test shows potential for indexing, it is unlikely to be used widely as a diagnostic tool.

The first two tests mentioned, both utilizing diffusion of antigen and antibody through an agar medium, offer perhaps the best possibilities for routine field diagnosis. The single diffusion, or radial immunodiffusion test is performed in plastic Petri dishes or on glass slides. The antiserum is incorporated into the agar, and the virus is placed in wells punched in the agar. As the virus diffuses from the well, it reacts with the antibody and forms a visible precipitate. This test has been applied by Shepard and Secor (1969) for indexing large numbers of potato tubers for potato virus X and more recently by Slack and Shepherd (1975) for indexing barley seeds for barley stripe mosaic virus (BSMV). Richter and Proll (1975)

used radial immunodiffusion for quantitative determination and mass testing of cucumber mosaic virus from several naturally infested hosts. Although use of this technique has not yet been reported with legume viruses, bean pod mottle virus has been detected from soybeans by the radial immunodiffusion method (Tolin, unpublished). This test has the advantage of accommodating a large number of samples simultaneously and thus is quite useful for indexing. Rather large quantities of antiserum may be needed, since it is incorporated into the agar in amounts depending on its titer. The sensitivity is very high. Slack and Shepherd (1975) detected about 1 μ g BSMV/ml, approximately a 10-fold greater sensitivity than conventional double diffusion tests. The virus concentration in field-grown legume tissue is usually far greater than the minimum level of detection. Inability to detect virus may be related more to improper proportions of antibody to antigen rather than to insufficient virus.

An equally satisfactory method for detecting virus from crude sap, clarified extracts or purified preparations is the Ouchterlony agar double-diffusion test. Antiserum and virus are added to wells punched in agar which is placed in plastic Petri dishes or on glass slides. The wells are arranged so that the serum is in a center well surrounded by peripheral wells containing the antigen. Antibodies and virus both diffuse into the agar and precipitin zones develop where they meet. The ratio of antigen to antibody must be such that the precipitin band forms between the wells rather than within either well. Sera that react slightly with proteins from healthy plants as well as with virus can be used since their precipitation lines rarely coincide. Controls of healthy sap and normal serum should always be included to show the location of any non-specific bands. A positive control known to

react with the antiserum is desirable. Modifications can be made as follows.

and concentration of agar, the preservative, the ionic constituency of the medium, and other additives to give conditions suitable for the development of specific precipitin bands for most legume viruses

The remainder of this presentation will be devoted to a discussion of the agar double-diffusion procedure for the diagnosis of both spherical and rod-shaped legume viruses. Much of this information is based on experimental data from the author's laboratory using sap extracted from greenhouse and field grown legumes, as well as purified virus preparations. No attempt is made to provide a comprehensive review of other works.

Spherical legume viruses are relatively easily detected by this procedure. The well-characterized viruses can be divided roughly into three groups based on their stability. Alfalfa mosaic virus (AMV), and members of the Bromovirus group - broad bean mottle (BBMV), cowpea chlorotic mottle (CCMV) and bean yellow stipple (BYSV) - and of the Ilarvirus group - tobacco streak virus (TSV) - are relatively labile viruses, sensitive to high ionic strength and/or pH. Each virus attains a high concentration in infected tissue, is easily purified in large quantities, and is a good antigen in rabbits, inducing a high-titered specific antiserum. Strong precipitin bands develop in gels within 36-48 hrs if the proper ratio of antibody to antigen is placed in the reactant wells.

The second group includes the Comoviruses - cowpea mosaic (CPMV) and bean pod mottle (BPMV) viruses, as well as southern bean mosaic (SBMV) and tobacco ringspot (TRSV) viruses. These virus capsids are more stable, and all but SBMV form empty capsids upon infection. They are easily purified, are good antigens in rabbits, and produce strong precipitin bands in gels

*to my experience this method resulted in large amounts of non specific
reactions under various conditions*

The third group includes the legume-infecting Cucumoviruses - certain strains of cucumber mosaic (CMV) and peanut stunt virus (PSV). The capsids of these viruses are sensitive to salt, detergents, and a number of other agents. Thus they are often difficult to purify even though they reach rather high concentrations in host tissues. They are poor antigens and are generally thought to dissociate in rabbits unless stabilized by pretreatment with formaldehyde (Francki and Habili, 1972). The titer of the antiserum produced is rarely high. The type of agar, ionic conditions, and ratio of antigen to antibody are all critical to the formation of a precipitin band distinguishable from the healthy sap and normal serum controls. Some strains are more stable than others and can be detected readily from crude sap using the double-diffusion method. Once conditions are established for any one virus-host system, the method is very reliable. In gels prepared from 0.8% Ionagar No. 2 or Agarose, containing 0.1% sodium azide, we have detected PSV from field grown peanut (Arachis hypogaea), bean (Phaseolus vulgaris) and tobacco (Nicotiana tabacum) (Tolin, unpublished). A dilution of serum (8-fold) was required in the center well, and a volume of only 0.02 ml was sufficient in 5 mm diameter wells to detect as little as 0.02 mg PSV/ml. Even though the serum contained some antibodies to healthy proteins, the precipitin reaction bands could be distinguished easily. Samples positive by serology were also positive by electron microscopy and mechanical transmission. ✓

A large number of viruses infecting legumes are flexuous rods belonging to the Potyvirus, Potexvirus or Carlyvirus groups. These viruses, particularly the Potyviruses, are prevented by their size and shape from diffusing through agar gels. Purification of viruses for antibody production is more difficult than with spherical viruses, but the viruses are relatively good antigens.

Antiserum produced upon injection of intact virus rods into rabbits also reacts specifically with dissociated or soluble protein subunits. The titer of the serum to free protein is much less than that to virus particles (5 or more 2-fold dilution steps less). The titer to free protein may be increased in serum by using the dissociated virus to inject the rabbits, but specificity is then greatly decreased. The resulting antibodies may react with several viruses within the Potyvirus group (Shepard et al, 1974). Such antisera are useful in determining distant relationships between viruses, but perhaps have less value in routine diagnostic studies.

A number of reagents have been employed to dissociate virus particles to free protein subunits that can diffuse through the agar and react with antibodies. Some of these include high pH (Purcifull and Shepherd, 1964, Purcifull and Gooding, 1970, Langenberg and Bill, 1972) and detergents (Hamilton, 1965, Shepherd and Grogan, 1967, Gooding and Bing, 1970). The dissociation can be done either prior to addition to the well, or reactants can be incorporated into the agar so that dissociation occurs within the agar gel. Problems may arise because reagents used to dissociate the virion may also affect the antibodies and cause non-specific precipitation.

Most of the dissociation procedures were attempted in our laboratory for the detection of Potyviruses of tobacco, soybean (Glycine max) and peanut in crude sap of field and greenhouse grown plants. The antisera were first checked against purified virus by the microprecipitation test to determine the specific titer, and then tested in the dissociating medium. The most reliable results with tobacco viruses were obtained with the method of

Gooding and Bing (1970) in which 0.5% of the detergent sodium dodecyl sulfate (SDS) is incorporated into the 0.8% Ionagar and 1% sodium azide. This method was also preferable because the extracted sap required no additional treatment, and sharp precipitin bands developed overnight. Soybean mosaic virus (SMV) was usually detectable in soybean sap by this method. Peanut mottle virus (PMV) in pea (Pisum sativum), peanut and soybean developed faint precipitin bands but considerable non-specific precipitation often occurred. The intensity of the specific and non-specific bands with both PMV and SMV varied greatly with the type and concentration of agar; the SDS concentration, and the ionic conditions of the medium. The addition of sodium chloride to the medium eliminated much of the non-specific precipitation. The medium in which most consistent results have been obtained is composed of 0.6% Ionagar No. 2, 0.2% SDS, 0.7% NaCl and 0.1% NaN_3 (Iolin and Roane, 1975). The increased salt evidently decreases the interaction between SDS and serum proteins which results in non-specific precipitin bands (Palmer et al, 1971, Cho and Feng, 1974).

We have found it necessary, however, to alter this medium with different lots of antiserum, but slight changes ($\pm 0.2\%$) in NaCl or SDS concentration has been adequate. Thus far, we have found conditions for each lot of serum and virus tested, including SMV, PMV, bean common mosaic (BCMV) and bean yellow mosaic (BYMV) viruses from soybean, pea, peanut, and bean (Phaseolus vulgaris). Gooding (personal communication) has also adjusted his conditions for tobacco virus identification by varying SDS and salt concentrations.

Other investigators have also had success with the SDS-double diffusion procedure. Uyemoto, et al (1972) used SDS with BCMV and BYMV, but generally preferred treatment of sap with 0.3% SDS prior to adding it to the agar.

In my experience the method results in large amounts of non-specific

precipitation of the serum proteins by SDS and was not reliable

Provvidenti and Schroeder (1972) identified BYMV with SDS in gels, but later Provvidenti and Hunter (1975) treated extracts with SDS prior to adding them to the wells. Jones (personal communication) modified the procedures of Tolin and Roane (1975) by using 0.1M Tris-HCl, pH 9 buffer in the agar medium, and has successfully indexed hundreds of naturally infected red clover samples for BYMV.

It is apparent that the agar double-diffusion test is useful for identification of spherical and rod-shaped legume viruses. Conditions of the medium can be modified for each case to make the test reliable. The proper concentration of the antigen and antibody is essential. With rods about 0.05ml of undiluted serum is used per 5 mm well surrounded by 6-8 peripheral antigen wells. With spheres the same volume of serum is added, but prior dilution of 1:8 to 1:512 may be necessary. Sap extracted by grinding in a mortar and pestle with a small amount of water or buffer, or expressed directly from leaves is used. The concentration of virus in field-grown legumes should be sufficient to give a positive reaction if the antibody concentration is correct. SMV, PMV and BPMV have been detected from field-grown soybean leaves picked at intervals through the season to maturity.

The agar double-diffusion test is simple and requires no elaborate equipment other than a means to melt the agar and suction to remove the plugs of agar from the wells. It is readily adapted to field conditions and offers perhaps the best method for identifying legume viruses in the field.

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