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NOVEL METHODS FOR REARING AND TESTING EUROPEAN RED MITE (PANONYCHUS ULMI) WITH ACARICIDES IN THE LABORATORY

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ABSTRACT

Despite being a serious crop pest, European red mite (Panonychus ulmi) is considered a very challenging species to rear and test under laboratory conditions. Recent work has led to modified procedures for culturing P. ulmi in large numbers, and to two new bioassays, the 'microimmersion' (MI) and 'residual cell' methods, based on topical and residual exposure to acaricides respectively. Mites are reared on first-winter seedlings of cherry-plum (Prunus cerasifera), obtained from a commercial nursery and stored at 1-3°C prior to replanting in individual planting tubes. This greatly facilitates sampling and the early elimination of contaminant species, especially Tetranychus urticae. MI and residual bioassays both yielded consistent baseline data for three acaricides tested against adults of a susceptible P. ulmi strain, and were equally effective at diagnosing strong resistance to chlorpyrifos in a North American population.

INTRODUCTION

Despite its frequent abundance as a pest of horticultural crops, the European red mite, Panonychus ulmi, has achieved notoriety as a very challenging species to maintain and manipulate under laboratory conditions. This arises from its comparatively slow reproductive rate (c. 1.5 eggs per female per day; Herbert, 1981), and the ease with which P. ulmi cultures can be invaded and rapidly supplanted by contaminant species, especially by the two-spotted spider-mite, Tetranychus urticae. One consequence is that the incidence and nature of acaricide resistance in P. ulmi, although undoubtedly widespread (eg. Cranham and Helle, 1985; Leonard, 1992), is still poorly documented in comparison to T. urticae, prompting unwarranted (and possibly erroneous) assumptions that phenomena described for the latter also apply to P. ulmi

In this paper we describe an alternative approach to rearing P. ulmi, based on published methods but refined to maximise throughput while minimising the prospects and adverse consequences of contamination. We also report on the first application to P. ulmi of two bioassay methods originally developed and validated with T. urticae in mind. Comparative data are presented on the baseline responses of a susceptible strain to three unrelated chemicals (bifenthrin, chlorpyrifos and dicofol), and on the expression of resistance to chlorpyrifos in a multiresistant population.

REARING OF P. ULMI

Host plants

Four species of the genus Prunus (P. avium, P. cerasifera, P. persica and P. spinosa) were initially evaluated for the dual role as hosts for mite rearing, and as sources of leaves for bioassays. Although all four satisfied the first criterion, only P. cerasifera consistently produced leaves that were sufficiently large to encompass the internal arena of bioassay cells (see below), and sufficiently robust to survive in the cells for up to 72h without inflicting significant control mortality on test subjects.

One further requirement was a means of generating plants rapidly and on a year-round basis. This has been met by purchasing from a commercial grower (Oakover Nurseries, Charing, Kent), first-winter seedlings of P. cerasifera uprooted while in dormancy following periods of frost, and stored in a cold-room at 1-2°C prior to delivery to Rothamsted. On receipt, these are cut to a required length (c. 20cm including roots and stem), dusted if necessary with the fungicide benomyl (Benlate, 50% wt/wt; Dupont) to combat Botrytis infections, and then re-stored at 1-3°C until needed for planting.

Prior to planting, seedlings are dipped in a 1000 mg/l solution of clofentezine (Apollo, 500g AI/l; Agrevo) as a precaution against overwintering eggs of phytophagous or predatory mite species. They are then planted individually in growing compost inside plastic planting tubes (21cm x 3.5cm diameter, Ray Leach Single Cell Container Systems; Stuewe & Sons, Cornvallis, Oregon, USA), held in PVC racks (36 tubes per rack) with drainage holes at the bottom of tubes immersed in c. 1cm water, replenished daily. Racks are stored in a quarantine growth room under an 18h photoperiod (provided by a combination of fluorescent and tungsten lighting) at 26°C for an average of 6-8 weeks before being used for rearing or bioassays. Epicormic buds typically form and sprout within the first 2 weeks, and foliage expands fully during the remainder of this period.

Rearing regime

Rearing cages (45cm wide x 45cm deep x 70cm high) are constructed from perspex with a hinged door at the front for access and a rotary fan mounted behind a mesh grill in the rear wall for ventilation. These are again stored in a constant-environment facilty at 26°C with an 18h photoperiod provided by fluorescent and tungsten lighting. Each cage normally contains four plants held in a PVC rack and changed singly at weekly intervals on a four-week cycle. Each week a batch of at least 50 mites, comprising both sexes and all life-stages (excluding eggs), are transferred from the oldest to the newest plant using a fine sable-hair brush (size 5/O) under 5-fold magnification to avoid picking up contaminant species.

Both the plant-growing and mite-rearing regimes need continuous scrutiny to detect infestations of T. urticae as early as possible, when they can usually be eliminated effectively by discarding individual contaminated plants rather than a complete culture. Strains maintained in single cages using this protocol have consistently yielded at least 1000 surplus adults per week for bioassay purposes, without compromising the continuity of the stock culture. If required, yield can be enhanced further by increasing the number of plants per cage or, preferably, the number of cages dedicated to that strain.

BIOASSAY METHODS

Both microimmersion and residual cell bioassay methods are described in detail elsewhere (Dennehy et al., 1993). Here we present only an outline of these methods, highlighting modifications incorporated for P.ulmi testing.

Microimmersion bioassay

The microimmersion (MI) bioassay involves drawing batches of 10 adult female mites from leaves removed from a rearing cage into small pipette tips under vacuum pressure, and immersing them for 30s in 35ul of a test solution of either formulated or technical acaricide. At the end of the immersion period, mites are exhausted onto a filter paper disc when individuals physically-damaged during immersion are discarded from the bioassay. After 10-20s drying on the filter paper, treated mites are transferred to holding cells (constructed from three layers of perspex held together by rubber bands; Dennehy et al., 1993), and enclosed within an arena formed on the upper surface of a clean P. cerasifera leaf whose petiole extends outside the cell into a 3cm scintillation vial containing water. Fully assembled cells are stored for the required holding period on wet cotton wool in a plastic seed tray, and then disassembled to allow dead and alive mites to be removed from the arena and counted.

Residual cell bioassays

Residual tests employ holding cells identical to those used for the MI bioassay. In this case however, P. cerasifera leaves are dipped for 5s in the required concentration of formulated acaricide, and allowed to dry before being enclosed in the cells. Batches of 10 mites are then brushed from infested leaves and loaded into each cell. Cells containing test subjects are stored as before, and assessed in the same manner.

Mite strains and acaricides

The UKS strain of P. ulmi used for baseline determinations has been maintained at Rothamsted without acaricide exposure since its collection from an unsprayed orchard in Kent, S.E. England, in September 1990. Extensive testing has shown this to be fully susceptible to all chemicals except some organophosphorus compounds (but excluding chlorpyrifos). The multiresistant NYR strain, derived from several intensively-sprayed orchards in New York State in 1991, has also been maintained without exposure to acaricides.

Diluted formulations of bifenthrin (Talstar, 100g/l EC; DowElanco), chlorpyrifos (Dursban, 480g/l EC; DowElanco) and dicofol (Kelthane, 180g/l EC; Rohm and Haas) were prepared using distilled water. Technical samples of bifenthrin and chlorpyrifos were of 95% purity, or greater. Stock solutions of technical products in acetone were diluted further with distilled water containing 0.0125% Triton X-100 to achieve final concentrations of acetone (200ml/l), surfactant (0.1g/l) and the desired strength of acaricide.

Design and analysis of bioassays

Dose-response MI bioassays were performed with formulated acaricides representing three chemical groups: bifenthrin (a pyrethroid), chlorpyrifos (an organophosphate), and dicofol (a chlorinated biphenyl compound), and with technical material of the first two chemicals. Residual bioassays were conducted with the same three formulated chemicals. Tests involving 5-8 different concentrations of each chemical were replicated a minimum of three times. All bioassays were held for 72h post-treatment in continuous darkness at 24°C. Mites incapable of moving at least one body length (with prodding if necessary) were recorded as dead.

Since bioassay results proved very consistent over time, dose-response data for different replicates were pooled and subjected to probit analysis using the POLO computer program (LeOra Software, Berkeley, California) to obtain single probit lines for each product with each type of bioassay.

BIOASSAY RESULTS

Both the MI and residual methods yielded dose-response relationships showing a good fit to the probit model, and close agreement between replicates both within and between bioassays. Probit analyses of baseline data for UKS mites are summarised in Table 1.

MI bioassays with formulated acaricides

As with T. urticae (Dennehy et al., 1993), LC50 values for bifenthrin and dicofol did not differ significantly, but both these chemicals were significantly less active than chlorpyrifos. Hence the ranking of toxicities was chlorpyrifos > bifenthrin/dicofol, with LC50 values ranging from 35mg/l (dicofol) to 7mg/l (chlorpyrifos). In absolute terms, these figures were 3- to 7-fold higher than corresponding ones for a susceptible T. urticae population.

MI bioassays with technical acaricides

Toxicities of technical bifenthrin and chlorpyrifos showed the same ranking as for formulated products. Interestingly, both LC50s were c. 3.5-fold higher than corresponding ones obtained above, possibly due to constituents of the commercial formulations enhancing penetration of both chemicals through the mite cuticle. Slopes of fitted probit lines did not differ significantly between technical and formulated material of either acaricide.

Residual cell bioassays

The relative toxicities of formulated chlorpyrifos and dicofol accorded well with those for the MI bioassay. LC50s for chlorpyrifos were virtually identical with both methods (7.0 and 6.6mg/l for MI and residual tests respectively), and differences between those for dicofol (35 and 93mg/l) were only barely significant at the 95% level (Table 1). In contrast, LC50s for bifenthrin differed markedly, being 20-fold higher with MI (24mg/l) than with the residual assay (1.2mg/l). The reason for this discrepancy is still unclear, but

may relate to the behavioural activity of bifenthrin residues causing mites to abandon treated surfaces and starve as a consequence. Significantly, we noted no corresponding difference in LC50s for T. urticae - a more resilient species capable of surviving for longer periods without food (Dennehy et al., 1993). Such findings serve well to highlight the risk of extrapolating results between Tetranychid species.

TABLE 1. LC50 values* (95% confidence intervals in parentheses) and slopes of probit lines obtained from MI and residual bioassays against the UKS strain of P. ulmi.

Chemical	No. tested	LC50		Slope
(a) MI bioassays with formulated acaricides				
Bifenthrin	570	24	(16-33)	1.4
Chlorpyrifos	560	7.0	(4.1-11)	1.5
Dicofol	520	35	(28-45)	2.3
(b) MI bioassays with technical acaricides				
Bifenthrin	470	88	(50-130)	1.2
Chlorpyrifos	840	26	(15-38)	1.9
(c) Residual bioassays with formulated acaricides				
Bifenthrin	1380	1.2	(0.8-1.6)	1.1
Chlorpyrifos	790	6.6	(3.0-10)	1.5
Dicofol	530	93	(49-156)	0.9

* Expressed in mg/l active ingredient.

Expression of resistance to chlorpyrifos

Both methods proved very effective in diagnosing strong resistance to chlorpyrifos in the NYR population (Table 2). Although none of the three LC50 values in Table 2 differed significantly from each other, the higher figure for UKS mites after immersion in technical chlorpyrifos (Table 1) resulted in this resistance factor (93-fold) being lower than in either test involving formulated material (610- and 420-fold for MI and residual tests respectively). Levels of chlorpyrifos resistance reported here mirror closely those documented recently for a strain of T. urticae, also of North American origin (Farnham et al., 1992). Biochemical assays have implicated reduced sensitivity of acetylcholinesterase to inhibition by chlorpyrifos-oxon as the primary cause of resistance in both species (G.D. Moores, unpublished data), although the kinetic properties of enzymes in P. ulmi populations have not been investigated in detail.

TABLE 2. LC50 values*, slopes and resistance factors (RFs) for chlorpyrifos tested against the NYR strain.

Bioassay	No. tested	LC50	Slope	RF
MI Formulated	320	4300	1.6	610
MI Technical	441	2400	3.4	93
Residual	610	2800	1.9	420

* Expressed in mg/l active ingredient.

CONCLUSIONS

Resistance problems with P. ulmi will continue to intensify without prompt action to deploy chemicals more rationally in accordance with known cross-resistance characteristics (eg. Leonard, 1992). By providing a continuous supply of mites for experimentation, and accurate means of detecting, selecting and characterising resistance in the laboratory, methods outlined in this paper should help considerably with accumulating the data on which sound resistance management tactics must be based.

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MANAGING POTATO CYST NEMATODE (*GLOBODERA PALLIDA*) IN INTENSIVE POTATO CROPPING SYSTEMS

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ABSTRACT

From 1983 to 1991 an experiment at ADAS Terrington tested how intensively potatoes could be cropped in land infested with *G. pallida* by using all the appropriate measures available to a commercial grower to manage the potato cyst nematode (PCN) population levels. These were:- tolerant or partially resistant cultivars, nematicide, rotation and soil fumigation, in various combinations. Growing potatoes every year in the same infested land could not be sustained profitably by the combined use of a nematicide (aldicarb), soil sterilisation (1,3 dichloropropene) every three or four years and the most resistant cultivar available (Sante). Cropping in alternate years improved the profitability of potato production but growing potatoes once in four years gave the best return of the management systems tested. The mean yield and mean pre-planting PCN soil population levels were smallest and greatest, respectively, in the continuous cropping system compared with the others tested.

INTRODUCTION

The two species of potato cyst nematode (PCN), *Globodera pallida* and *G. rostochiensis*, are the most important pests of potatoes in the UK (Hancock, 1988) and growers need to manage them effectively in order to grow profitable crops in infested land. The incidence of *G. pallida* has increased with the growing of cultivars resistant to *G. rostochiensis* in land infested with both species but the biology of *G. pallida*, plus the lack of totally resistant cultivars, makes management of this species potentially difficult. The experiment described here started with the simple aim of testing the best commercially available management practices for growing potato crops intensively in land infested with PCN. *G. pallida* quickly became the predominant species and the treatments evolved to meet this challenge, as a grower would have to react in a commercial enterprise.

MATERIALS AND METHODS

The experiment started in 1983, with two preliminary, pre-treatment years, and finished in the autumn of 1991. It was sited at ADAS Terrington, near King's Lynn, Norfolk, on a silty loam soil (Wisbech Series).

Design and treatments

The design of the experiment was not conventional, being a randomised block design with partial, uneven replication. There were 12 plots arranged in 4 blocks. Each plot was

42 m long by 16.2 m wide and separated from adjacent plots by a minimum of 5 m of uncropped land. The main treatments were 1) potatoes grown every year, 2) potatoes grown every other year, alternating with a cereal crop, and 3) potatoes grown once every four years in the sequence potatoes, cereal, sugar beet, cereal, potatoes, etc. Each treatment was represented once in each block but phased so that in any one year all plots of Treatment 1, two plots of Treatment 2 and one plot of Treatment 3 were cropped with potatoes. In 1984 and 1985 the potato cultivar Cara was used throughout the plots because it was a high yielding maincrop cultivar resistant to *G. rostochiensis* and very tolerant of attack by both species of *Globodera*. In 1986, the plots were split equally to enable a comparison of Cara with Sante, first available in the UK in 1985 as a high yielding maincrop cultivar resistant to *G. rostochiensis* and partially resistant to *G. pallida*. In 1987, Sante was compared with Kingston, a very high yielding maincrop cultivar resistant to *G. rostochiensis*. From and including 1988, Sante was the only cultivar grown but the plots were split equally with one subplot treated with aldicarb at the recommended rate (Temik, 33.6 kg/ha) and the other untreated.

In autumn 1984, 1987 and 1990, plots were fumigated with the partial fumigant 1,3-dichloropropene (Telone II, 225 l/ha), applied by a contractor using a sub-surface injector combined with a soil-sealing roller. In 1984, all the plots and subplots of Treatment 1 and one of the two subplots of every plot in Treatment 2 were treated. In 1987 and 1990, all plots were treated overall.

Crop husbandry

All operations were done using standard farm equipment and methods. No irrigation was applied throughout the experiment. Control of weeds, pests other than PCN and late blight followed the usual farm programme. Fertiliser rates used were: 1983 - 220 kg/ha N, 250 kg/ha P_2O_5 and 250 kg/ha K_2O; 1984 and 1985 220:220:220; 1986 - 250:220:220. From 1987 to 1991 inclusive, the plots of Treatment 1 received 170:80:240 (170:60:240 in 1989) and those of the other treatments growing potatoes 250:250:250. Rates applied to Treatment 1 were based on the results of nutrient analysis of soil samples.

Assessments

Before planting and after harvest every year, the soil of each subplot cropped with potatoes was sampled by taking 50 cores (each approximately 2 cm wide by 22 cm deep) with a cheese corer and the number of live PCN eggs per gram (e/g) of soil in each subplot determined using standard techniques. The identity of the PCN species present at the site was determined using iso-electric focussing in 1984, 1986 and 1991. At harvest the yield of potato tubers from a total of 15 m of row in each subplot was weighed by grade size. Results for yield and nematode assessments were subjected to analysis of variance and regression analysis, as appropriate, using the GENSTAT and Minitab programs.

RESULTS

The design of the experiment was such that comparisons between treatments and years must be made with caution. Results of particular importance are detailed below. In 1985, *G. pallida* was the predominant species in about 20 % of the plots tested. In 1986 and 1991, *G. rostochiensis* was not detected.

Effect of rotation on PCN

The success of managing the nematode populations over the life of the experiment can be crudely judged by the pre-planting levels in 1991, in which the means were 21.9, 8.5 and 2.5 e/g for continuous, 1 in 2 and 1 in 4 rotations, respectively. This was after soil fumigation and with cropping with the best resistant cultivar. The effect of rotation is demonstrated more clearly by the mean pre-planting levels in 1990; 61.9, 34.5 and 4.5 e/g for the continuous, 1 in 2 and 1 in 4 rotations, respectively. Taking the most complete sub-set of data, that for Sante 1988 to 1991 inclusive (Table 1), the mean pre-planting levels were; 97.6, 37.4 and 17.8 e/g (SED 27.0) for the continuous, 1 in 2 and 1 in 4 rotations, respectively. These results suggest that a substantial level of PCN must be accepted as a consequence of close cropping, especially if fumigation is not possible or not used before every crop (routine fumigation was not tested in this experiment).

Effect of cultivar and nematicide on PCN

Two of the three cultivars used were grown with the nematicide aldicarb only. The mean Pf/Pi (post-harvest nematode level/pre-planting level) was 4.39 (SE 0.56) for Cara in 1986 and 3.54 (SE 1.45) for Kingston in 1987, both with aldicarb. For a totally resistant cultivar the Pf/Pi would generally be expected to be about 0.3-0.4; for a susceptible cultivar with a nematicide about 2.0 to 5.0, rising up to about 50 without a nematicide. Each cultivar performed as would be expected for a susceptible cultivar with a nematicide. The partial resistor Sante was grown with and without nematicide. From 1988 to1991, regardless of nematicide use and rotation, the mean Pf/Pi for Sante was 1.31 (SE 0.18). Using a nematicide reduced ($P<0.05$) the multiplication (Pf/Pi) of PCN from 1.69 (without nematicide) to 0.93 (with nematicide) and resulted in a lower mean post-harvest PCN level ($P<0.05$) (45.5 e/g) than without a nematicide (72.6 e/g). The results suggest that a nematicide needs to be used with Sante to gain the maximum effect from its partial resistance and maintain the population at no more than the pre-planting level. The linear regressions of best fit of Pf on Pi were:-

1986	Cara + ald.	$Log10(Pf) = 0.89 + 0.82Log10(Pi)$; r-sq = 87.7%; $P<0.001$
	Kingston + ald.	$Log10(Pf) = 1.46 + 0.39Log10(Pi)$; r-sq = 79.8%; $P<0.001$
	Sante + ald.	$Log10(Pf) = 0.79 + 0.52Log10(Pi)$; r-sq = 59.2%; $P=0.001$
1987	Sante + ald.	$Log10(Pf) = 0.81 + 0.56Log10(Pi)$; r-sq = 63.0%; $P<0.001$
1988-91	Sante + ald.	$Log10(Pf) = 0.95Log10(Pi) - 0.09$; r-sq = 61.9%; $P<0.001$
	Sante - ald.	$Log10(Pf) = 0.67 + 0.61Log10(Pi)$; r-sq = 55.6%; $P<0.001$

Effect of fumigation on PCN

Fumigation was used on three occasions during the experiment. The records were incomplete for the split fumigation in 1984 and could not be considered further. In 1987 and 1990, and omitting the few anomalous values where pre-treatment levels were very low, the mean Ppost/Ppre (post-treatment nematode level/pre-treatment level) was 1.23 and 0.39, respectively. Comparison of the Ppost/Ppre values for all plots showed that the effect of fumigation differed between the two years ($P<0.001$, Mann-Whitney). The linear regressions of best fit of post-treatment on pre-treatment levels were:-

1987 Log10(Ppost) = 0.934 + 0.477 Log10(Ppre), r-sq = 64.1%, P<0.001
1990 Ppost = 5.18 + 0.265 Ppre, r-sq = 25.0%, P<0.01

where Ppre = PCN level pre-treatment in e/g and Ppost = PCN level post-treatment in e/g.

Theoretically, if the fumigation worked perfectly, there would be no viable eggs and, therefore, Ppost would be independent of Ppre, ie, b = 0. It is possible that the regression coefficient b is a fumigation efficiency factor, influenced by soil temperature and moisture content, and exposure time of the target to the fumigant. The constant "a" suggests a residual population of potato cyst nematodes that could be related to the effect of post-treatment cultivation in bringing up viable cysts from below the fumigated layer and could be linked to the size of the pre-treatment nematode population, which in 1990 was much larger (mean 107.3 e/g) than in 1987 (41.1 e/g). Overall, it seems that fumigation gave better control of PCN in 1990 than in 1987.

TABLE 1. Mean pre-planting (Pi) and post-harvest (Pf) levels of PCN, Sante 1988-1991

	Pi (eggs/g soil)				Pf (eggs/g soil)			
Rotation	1988	1989	1990	1991	1988	1989	1990	1991
1. 1/1 rotation								
Sante + aldicarb	108.5	136.6	51.1	26.6	136.6	51.1	26.2	23.0
- aldicarb	147.4	220.8	72.6	17.1	220.8	72.6	17.1	26.8
2. 1/2 rotation								
Sante + aldicarb	60.2	37.6	24.8	5.8	43.8	14.6	32.8	4.4
- aldicarb	72.5	42.8	44.2	11.1	109.6	35.4	38.6	13.6
3. 1/4 rotation								
Sante + aldicarb	34.5	19.0	4.3	(3.4)	23.2	7.8	0.1	(-)
- aldicarb	37.2	37.5	4.6	1.6	89.5	31.8	36.9	3.9

(In 1991 aldicarb was not applied to any plot of Treatment 3 although inclusion above of the pre-planting level is valid.)

Yield

In 1985, the mean total and saleable ware yields of Cara in the continuous rotation were 52.5 and 40.0 t/ha, respectively. In the 1 in 2 rotation the corresponding mean yields were 52.8 and 39.5 t/ha (fumigated) and 41.1 and 29.2 t/ha (unfumigated). In the 1 in 4 rotation, the mean yields (51.3 and 39.8 t/ha, respectively) were comparable to those obtained in fumigated treatments. However, in all treatments there was not a close relationship between yield and pre-planting nematode level, suggesting that factors other than the nematode were also important in limiting yield.

In 1986, the mean yields of Cara were generally lower than in 1985. Total and saleable ware yields were:- continuous - 30.4 and 26.7 t/ha, respectively; 1 in 2 - 31.9 and 28.8 t/ha, and following fumigation in 1984, 42.0 and 37.4 t/ha; 1 in 4 - 44.8 and 42.3t/ha. The yields of

the other cultivar, Sante, were comparable with Cara; continuous - 32.2 and 23.9 t/ha; 1 in 2 - 35.6 and 28.8 t/ha, and following fumigation in 1984, 52.8 and 43.8 t/ha; 1 in 4 - 47.3 and 36.7 t/ha. Fumigation in 1984 seemed to improve yield from the 1 in 2 rotation in 1986 greatly although the PCN levels were appreciably higher (mean 47.3 e/g) than in the 1 in 4 rotation (mean 1.6 e/g).

In 1987, the PCN levels in the continuous cropping were very high (Sante 103.2 and Kingston 368.3 e/g) and the yields reflected this; Sante mean total and ware yields were 16.0 and 8.6 t/ha, and Kingston, 9.8 and 4.3 t/ha. Both cultivars had much lower yields in the continuous rotation than the other two ((P<0.001).

TABLE 2. Mean ware yield (t/ha) of Sante with and without aldicarb (fumigation in 1990).

Rotation	Continuous		1 in 2		1 in 4	
Year	+ ald.	-ald.	+ ald.	- ald.	+ ald.	- ald.
1988	30.3	31.1	38.4	37.2	38.9	28.7
1989	15.6	11.8	30.0	30.7	40.3	31.4
1990	6.0	5.5	11.6	5.6	19.2	14.2
1991	35.1	32.0	34.3	32.9	-	44.9
Mean	21.7	20.1	28.6	26.6	32.8	24.8

For 1988-1991, the saleable ware yields (45 -85 mm) (Table 2) generally reflected the total yields. The use of aldicarb improved yields in some years (total yield in 1989 (P<0.01), total and ware yields in 1990 (P<0.05)). Lengthening the rotation sometimes improved yields: in 1988 the 1 in 2 rotation gave the best ware yield (P<0.05); the total yield in 1989 and 1990 and the ware yield in1989 were worst in the continuous rotation (P<0.01) and better in the 1 in 4 (P<0.05) than the 1 in 2; in 1990, the ware yield in the 1 in 4 was best (P<0.01). Uneveness of performance may have been related to the Pi levels although there was only a weak relationship between yield and Pi.

Economic performance

Taking the best possible practice in the experiment to control PCN, that is, Sante with a nematicide plus fumigation in 1987 and 1990, and costs and values from Nix (1992), the gross margin of the saleable ware (1986-1991) was considerably improved by lengthening the rotation (P<0.01) (means - continuous rotation £327/ha, 1 in 2 £1078/ha and 1 in 4 £1449/ha, SED £294/ha). In 1987, 1989 and 1990 variable costs in the continuous rotation exceeded revenue.

DISCUSSION

The control of *G. rostochiensis* using integrated management, combining rotation, resistant cultivars and nematicide plus the occasional fumigation to reduce very high PCN

levels when needed and possible, is widely used in the UK and enables growers to follow sustained, intensive rotations. Problems arise when *G. pallida* is also present in the soil and its population increases when competition from *G. rostochiensis* is reduced by control measures that are less effective against *G. pallida*. This happened in the first few years of this experiment. The results of the experiment suggest that economically viable potato cropping can probably be sustained when production is spread over a 1 in 4 rotation and a partially resistant cultivar plus nematicide is used. Trudgill *et al.* (1992) recommended that partially resistant cultivars were most useful at low Pi's and must be used in an integrated system (eg, with rotation or reduced rates of nematicide) to prevent populations increasing appreciably. Experience with Sante in this experiment confirms that a nematicide is almost certainly necessary and even then the PCN population may still increase.

The results suggest that it may be possible to follow a profitable 1 in 2 rotation for some years but fumigation or a break from cropping will be needed eventually. They also indicate that soil fumigation with 1,3 dichloropropene can have a variable effect on PCN levels, which may be related in part to the distribution and, perhaps, size of the PCN population treated and suggest that fumigation should not be relied on to rescue a grower from bad management of PCN. The experiment did not test the routine use of frequent fumigation as has been used in some other countries. Interestingly, it seems that fumigation can affect yield beyond the crop immediately after treatment although the Pi's in the experiment indicate that such an effect is not directly linked with PCN.

Overall, the results of the experiment show that it is possible to crop potatoes profitably in land infested with *G. pallida* but following a rotation shorter than 1 in 4 requires very careful management and may not be sustainable in the long-term. The grower will also need to consider the overall economic performance of the rotation and not just the potato component.

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A STRATEGY FOR MAINTAINING EFFECTIVE HOST-PLANT RESISTANCE TO POTATO CYST NEMATODES

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ABSTRACT

Recent field and experimental observations have revealed a breakdown in the resistance of potato cultivars to populations of the pale potato cyst nematode (PCN), *Globodera pallida*. The selection of nematode virulence genes would appear to be the cause of the observed "loss of resistance" and this, combined with the relatively narrow range of resistance genes present in currently available cultivars, has led to concern over their long term effectiveness. Investigations are currently identifying and characterising novel PCN resistance in a range of wild and cultivated *Solanum* species and incorporating that resistance into a commercial potato breeding programme. This will form the basis of an effective management regime for PCN populations in field conditions.

INTRODUCTION

Several control options exist to manage potato cyst nematode (PCN) populations within standard potato production practices. In temperate regions, the usual methods are either extended crop rotation with non-hosts, chemicals (nematicides), resistant varieties or a combination of these methods. Generally, the most acceptable method makes use of resistant varieties and there now exist many potato varieties with resistance to PCN.

Unfortunately, resistance in most varieties is based on a very narrow gene pool, predominantly gene H_1 from *Solanum tuberosum* ssp. *andigena*, which confers high levels of resistance to *Globodera rostochiensis* (Ro1/4) but is ineffective against all other pathotypes (Toxopeus and Huijsman, 1953). Resistance to all pathotypes of both PCN species (*G. rostochiensis*, *G. pallida*) was first identified in the wild diploid potato species *S. vernei* (Ellenby, 1948) which operates by a complex of major and minor genes (Ross, 1969) and has been the basis for introducing *G. pallida* resistance into several potato varieties. Recent evidence from both field and experimental observations has now revealed a breakdown of resistance in potato cultivars to *G. pallida* populations, as virulent individuals within the populations are selected by repeated use of the same resistance source.

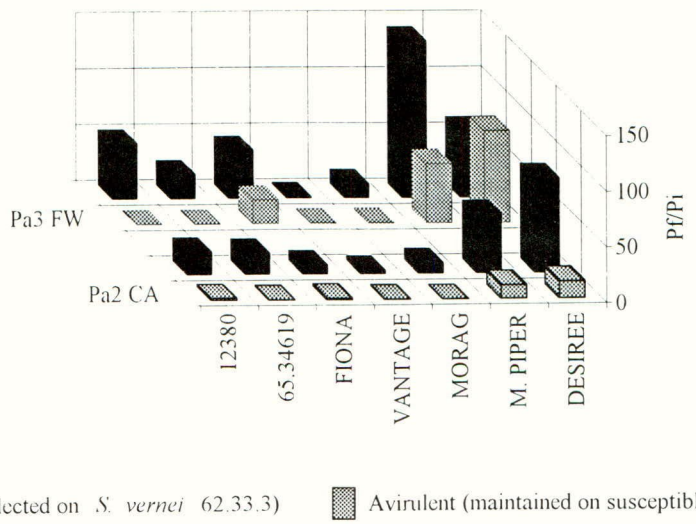
In order to fully exploit the wide range of resistance available within both wild and cultivated potatoes, the mechanisms of resistance must be fully understood and techniques for the accurate identification of virulence genes within PCN field populations developed. Once achieved, a strategy for maintaining effective host-plant resistance to PCN can be implemented.

SELECTION OF VIRULENT *GLOBODERA PALLIDA* POPULATIONS

Repeated inoculation of *G. pallida* populations onto potatoes containing resistance genes from *S. vernei* (62.33.3 and 65.346/19) selected those individuals within the populations containing virulence genes and able to reproduce on such clones (Turner, 1983). Continued selection identified these virulent populations as genetically distinct and as equally fit as their unselected counterparts at reproducing under field-type conditions (Turner, 1990).

Additional studies have shown that these virulent populations also have enhanced reproductive ability on a range of commercial potato varieties whose PCN resistance is based on *S. vernei* (Figure 1). All virulent populations of Pa2 Cadishead (CA) had significantly higher reproduction rates than their avirulent counterparts ($p < 0.05$ - 0.001) as did most Pa3 Frenswegen (FW) virulent populations. Only the Frenswegen populations on Fiona, Vantage and Desiree showed no significant enhancement. Field data from eastern England confirms that the partial *G. pallida* resistance of Sante can break down by its repeated use, probably due to selection of virulent populations.

FIGURE 1. Multiplication rates (Pf/Pi) of virulent and avirulent *G.pallida* populations on a range of potato cultivars.



It is now appreciated that the mechanisms of PCN resistance differ between potato species (Hoopes, 1977), thus virulent populations selected on one PCN resistant source

(e.g. *S. vernei*) may be avirulent on another (e.g. *S. multidissectum*) (Turner, 1990). A potential management tool therefore exists to manipulate field populations by altering the resistant source used to manage them.

IDENTIFICATION OF NOVEL SOURCES OF PCN RESISTANCE

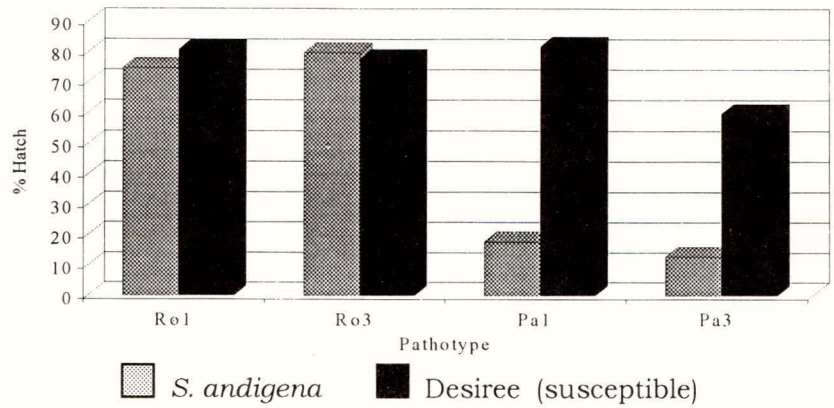
In order to broaden the genetic basis of PCN resistance, accessions were obtained from the Commonwealth Potato Collection and evaluated for resistance to European populations of PCN (Turner, 1989). Resistance to both *Globodera rostochiensis* and *G. pallida* was identified from 51 accessions in 16 species from Argentina, Bolivia and Peru. This has formed the basis of on-going investigations to clarify the differences between potato species on the basis of their PCN resistance.

The life-cycle of PCN was divided into distinct stages in order to study where resistance was operating i.e. hatching stimulation, root invasion, development to adulthood within the root system, and reproduction. In addition, differences in the feeding sites (syncytia) are currently being observed.

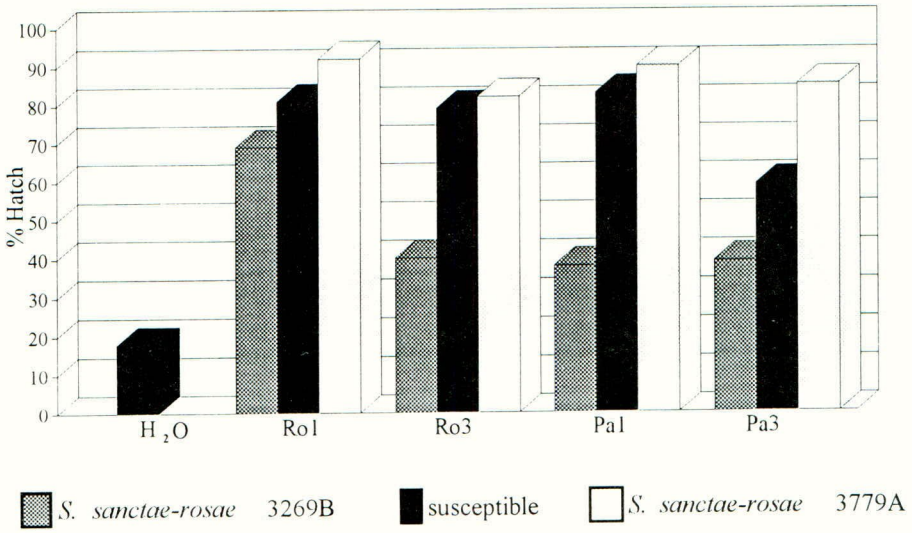
Studies to date have concentrated on hatching mechanisms and invasion ability. Standard hatching tests with root exudate, (Widdowson, 1958) were conducted on 11 species over a 6 week period. Compared to both a susceptible control exudate (Desiree) and water, significant differences in both stimulation and inhibition of PCN hatch occurred. For example *S. andigena* 591B produced strongly suppressed hatch of *G. pallida* populations but stimulated hatch of *G. rostochiensis* (Ro1 & Ro3) (Figure 2A). Accessions within a potato species were also capable of a range of responses e.g. *S. sanctae-rosae* stimulated hatch with 3779A exudate but inhibited it with 3269B exudate (Figure 2B). In conclusion, significant differences in both stimulation and inhibition of PCN hatch were observed but there was no consistent correlation between the resistance ratings of the potato clones and their ability to stimulate or inhibit hatch.

FIGURE 2. % hatch of potato cyst nematode juveniles by potato root exudate.

(a) Solanum andigena 591B



(b) Solanum sanctae-rosae Accessions 3269B & 3779A



Studies on root invasion rates, using hatched PCN juveniles, generally confirmed the original resistance rating i.e. those clones suppressing hatch were still at least partially resistant when hatched juveniles were inoculated onto their root system (Fleming & Turner, unpublished). Therefore, although both root exudate and invasion ability may be modifying PCN reproductive rate they do not appear to be the main mechanisms of resistance.

Previous investigations indicated that PCN resistance operates mainly after root invasion, by the nematodes failing to establish an efficient syncytium and thus affecting development to adulthood (Roberts & Stone, 1983; Turner, 1984). Current investigations are clarifying if this is so with the additional sources of resistance now identified.

INCORPORATION OF PCN RESISTANCE INTO A COMMERCIAL BREEDING PROGRAMME

PCN-resistant clones from 10 potato species, and representing the highest levels of resistance, are currently being incorporated into a commercial breeding programme. The example of *S. sparsipilum* is typical (Figure 3). The resistant source is normally used as the male parent and, if diploid (2n), the susceptible di-haploid *S. tuberosum* ssp. *tuberosum* is used to enhance a successful cross. Screening of the F_1 seedling progeny has confirmed the polygenic nature of resistance which has started to segregate at the initial cross (Table 1). Large numbers of crosses will be required to increase the chances of broad-based resistance being inherited. However, hybrid vigour has been observed in the F_1 seedlings (e.g. L3334/6) (Table 2).

FIGURE 3. Breeding programme and assessment of PCN- resistant potatoes (example *S. sparsipilum* CPC 3562D)

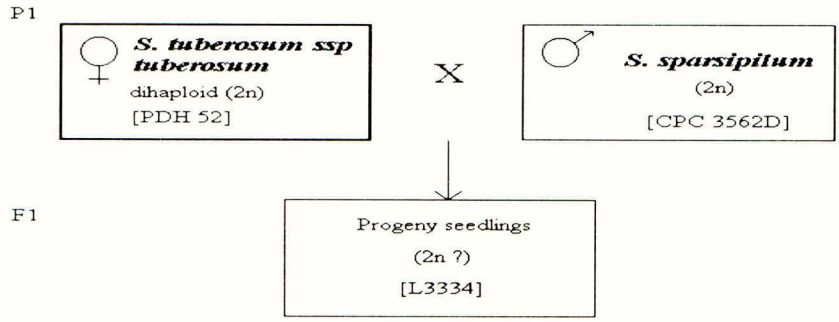


TABLE 1. Multi-pathotype PCN resistance of CPC F_1 seedling (ex. *sparsipilum*) compared with *S. sparsipilum* parent (CPC 3562D)

		Resistance to PCN pathotypes					
		Ro1	Ro2	Ro3	Ro5	Pa1	Pa2/3
Parent:	*S. sparsipilum*	R	R	R	R	R	R
F_1 progeny	L3334/1	R	--	--	--	--	--
	L3334/2	R	--	--	--	--	--
	L3334/3	R	--	R	--	R	--
	L3334/4	R	--	R	--	--	--
	L3334/5	--	--	--	--	--	--
	L3334/6	R	--	R	--	--	--

-- = susceptible/partial resistance

TABLE 2. Agronomic performance of F_1 seedling L3334/6

	Yield ($t.ha^{-1}$) for tuber size(mm) fraction						Total yield as % P.Crown	Tuber score 1=poor, 5=excellent
	>80	80-60	60-40	<40	Total	(SE)		
Maris Piper	1.40	20.20	21.10	3.00	45.70	(6.4)	98	2
Pentland Crown	7.20	30.00	9.00	0.60	46.80	(3.4)	100	4
L3334/6	20.10	29.40	7.60	0.20	57.30	(4.8)	122	3

MANAGEMENT STRATEGY TO MAINTAIN EFFECTIVE PCN RESISTANCE

- PCN-resistant varieties should be bred from a wide range of PCN-resistant potato species.

- PCN-resistant varieties should be bred from PCN-resistant potato species whose exudate stimulates high nematode hatch since this will increase decline rates.

- Farmers should be advised to alternate their use of PCN-resistant varieties to reduce the chance of selecting virulent nematode populations.

- PCN field populations should be regularly monitored to ensure that virulent populations have not been selected through the repeated use of one resistance source.

- Techniques should be developed to identify PCN populations containing genes virulent to PCN-resistant potato cultivars.

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BEMISIA TABACI - POTENTIAL INFESTATION, PHYTOTOXICITY AND VIRUS TRANSMISSION WITHIN EUROPEAN AGRICULTURE

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ABSTRACT

Over the past decade *Bemisia tabaci* (Genn.) the cotton whitefly, has become a major pest to agriculture globally. Previously limited to a narrow host range of plants within various tropical and sub-tropical regions, a new polyphagous biotype, the "B" biotype, has evolved and is now appearing in more temperate regions, including southern Europe and within glasshouses of northern Europe. This biotype is shown to feed on and colonise many crops grown within Europe and in some cases induce phytotoxic responses. Some crops are also found to be susceptible to infection from whitefly-transmitted geminiviruses that are already endemic to other global locations.

INTRODUCTION

Whiteflies have always been associated with agriculture worldwide as pests of certain crops and have caused damage by feeding and by virus transmission (Markham *et al.*, 1994). Recently however, the cotton whitefly, *Bemisia tabaci* (Genn.) has, through the evolvement of a number of super strains or biotypes, become a primary pest of diverse crop species in many previous and new localities. One new strain has been called the "B" biotype, based on its characteristic esterase banding pattern (Bedford *et al.*, 1992; Bedford *et al.*, 1993). It has also controversially, been designated as a new species, *Bemisia argentifolii* (Bellows *et al.*, 1994), based on limited morphological differences found within *B. tabaci* populations from the Americas. The "B" biotype of *B. tabaci* exhibits an increased fecundity (Bethke *et al.*, 1991), a predisposition to develop resistance to most insecticides (Byrne & Devonshire, 1993) and an extremely broad host range (Wool & Greenberg, 1990) estimated to encompass around 600 plant species. The "B" biotype is also capable of inducing phytotoxic responses in certain plant species, such as squash silverleaf. This latter phenomenon distinguishes the "B" biotype from any other biotype of *B. tabaci* (Bedford *et al.*, 1994a).

All biotypes of *B. tabaci* are capable of transmitting many different plant viruses, including nearly 60 different geminiviruses (Markham *et al.*, 1994). However, the ability of the "B" biotype to feed and colonise a greater range of plant species, has meant that the viruses it transmits are no longer limited to the usual narrow host range of any particular vector population (Bedford *et al.*, 1994a). The "B" biotype presents a far greater risk to global agriculture since "new" virus infections can potentially occur in previously unaffected crops wherever this vector is present.

The "B" biotype has become established in areas of the north European glasshouse industry and is appearing in the field in southern Europe. For this study, we have chosen a range of field and glasshouse crops commonly grown in Europe. We have tested their susceptibility to "B" biotype *B. tabaci* infestation, associated phytotoxic responses, and to infection from any of six whitefly-transmitted geminiviruses already endemic to areas outside Europe. These geminiviruses are, (i) Asystasia golden mosaic (AGMV), from Africa, infecting *Asystasia gangetica*, a common weed species (Bedford *et al.*, 1992), (ii) Ageratum yellow vein (AgYVV-S), from Singapore infecting the common weed *Ageratum conyzoides* (Tan & Wong, 1993), (iii) tomato yellow leaf curl (TYLCV-Y) from tomato in the Yemen (Bedford *et al.*, 1994b), (iv) bean calico mosaic (BCMoV) from beans in

the Americas (Brown *et al.*, 1990), (v) watermelon chlorotic stunt (WCSV), a severe virus in watermelons from the Yemen (Bedford *et al.*, 1994b) and (vi) Sida golden mosaic (SiGMV-CR), from an indigenous shrub *Sida carpinifollia,* in Costa Rica (Bird, 1958).

MATERIALS AND METHODS

Origin, identification and maintenance of insects

B. tabaci were obtained from a glasshouse infestation on *Solanum nigrum* in Florida USA in 1991. They were maintained at the John Innes Centre on *S. nigrum* plants in a perspex cage (90cm x 45cm x 45cm) in a growth-room at 25°C with a 16h daylength. A morphological study of the 4th instar/puparium stages was made with a Cam Scan Series 4 scanning electron microscope, and compared to taxonomic keys (Martin, 1987) to confirm the species identification. Biotype identification was achieved by non-specific esterase marker analysis and by a squash silverleaf bioassay (Bedford *et al.*, 1993).

Insect survival, adaption to host crops and phytotoxic induction

A selection of field and glasshouse crops common to European agriculture (Table 2), were sown one per 3.5 inch plastic pot. At the first full leaf stage, 30 *B. tabaci* adults were caged onto each plant at 25°C with a 16hr daylength. The number of insects alive after 24, 48, 72 and 96 hours was recorded for each. This was compared to controls with 30 insects in empty cages. Plants were left for 4 weeks and observed for whitefly colonisation. Plants were also compared to whitefly-free control plants for any associated phytotoxic damage. Each test was replicated three times.

Viruses and virus transmission

Plants infected with whitefly-transmitted geminiviruses were either collected from the location where the virus is endemic, or obtained from other research centres (Table 1). All viruses were previously shown to be transmitted by *B. tabaci* to their original host plants (Bedford *et al.*, 1994a). *B. tabaci* were established as colonies on each of the virus infected plants. Groups of 50 viruliferous insects were removed and caged onto seedlings of the selected crop plants at 25°C with a 16hr daylength. After 48 hours, whiteflies were removed and the seedlings transferred to an insect proof glasshouse where they were fumigated with a carbamate-based insecticide (propoxur, Octavius Hunt Ltd.) and observed for virus symptom development.

Confirmation of virus infection

Where virus infection could not be ascertained by symptoms alone or by a further acquisition/transmission test, leaves were harvested and total nucleic acid extracted as described by Covey & Hull, (1981). Samples containing 5 micrograms of nucleic acid were electrophoresed in 1% agarose slab gels which were then blotted as described by Southern, (1975). Oligolabelled cloned DNA fragments of all of the tested viruses were used to detect their infection of a test crop plant except for SiGMV and BCMoV where Abutilon mosaic virus DNA was used.

RESULTS

Insect identification

A morphological study of the 4th instar/puparium stages of individuals from the Florida whitefly colony, showed them to have features typical of the species *B. tabaci,* when compared to

TABLE 1. Whitefly-transmitted viruses tested, their codes, origins and maintenance methods.

Virus	Code	Origin / Year	Source	Maintained*
Asystasia golden mosaic	AGMV	Benin 1989	R. Markham	(i)(v)
Ageratum yellow vein	AgYVV-s	Singapore 1993	J. Stanley	(i)
Tomato yellow leaf curl	TYLCV-y	Yemen 1989	P. Jones	(i)(v)(g)
Bean calico mosaic	BCMoV	Arizona 1992	J.K.Brown	(i)
Watermelon chlorotic stunt	WCSV	Yemen 1989	P. Jones	(i)(g)
Sida golden mosaic	SiGMV	Costa Rica 1990	R.Markham	(i)(v)

* (i) = insect transmitted, (v) = vegetative propagation, (g) = grafting.

TABLE 2. Mean survival figures for 3 replicates of 30 *Bemisia tabaci* per test plant after 24, 48, 72 and 96 hours, induced phytotoxic responses and potential to infest test plants within 4 weeks.

Crop Species	Insect survival 24h	48h	72h	96h	Phytotoxic response	Colonisation potential
CONTROL	1	0	0	0	-	-
Turnip	27	26	22	21	None	very high
Swede	27	25	22	18	None	very high
Cabbage	30	27	23	21	White stem/leaf yellowing	very high
Lettuce	30	28	27	25	Leaf distortion/yellowing	high
Carrot	26	21	13	13	Yellow veining	medium
Sugar beet	18	15	9	5	None	poor
Garlic	21	18	12	10	None	poor
Onion	12	5	3	2	None	poor
Pea	30	29	27	23	Leaf yellowing	high
Oilseed rape	20	20	20	18	None	very high
French bean	29	26	25	23	None	very high
Broadbean	29	26	21	18	None	medium
Cauliflower	27	24	19	18	Leaf yellowing	very high
Sweet pepper	29	26	24	22	None	high
Marrow	29	28	24	22	Silver leaf	high
Tomato	28	22	20	17	Fruit miss-ripening	low/medium
Potato	30	27	23	20	Leaf yellowing	very high
Cucumber	26	26	21	18	Yellow veining	very high

TABLE 3. Susceptibility of crops to infection by six whitefly-transmitted geminiviruses.

	Virus					
Crop	AGMV	AgYVV-s	TYLCV-y	BCMoV	WCSV	SiGMV
Turnip	-	+	-	-	-	-
Swede	-	-	-	-	-	-
Cabbage	-	NT	-	-	-	-
Lettuce	-	-	-	NT	-	NT
Carrot	-	-	-	-	-	-
Sugarbeet	-	NT	-	-	NT	-
Garlic	-	-	-	NT	NT	-
Onion	NT	NT	-	-	NT	NT
Pea	NT	-	-	-	-	-
Oilseed rape	-	NT	NT	-	NT	-
French bean	-	+	-	+	+	+
Broadbean	-	NT	-	NT	-	-
Cauliflower	-	-	-	-	-	-
Sweet pepper	NT	-	-	-	-	-
Marrow	-	-	-	-	-	-
Tomato	-	+	+	-	-	+
Potato	-	-	-	-	-	NT
Cucumber	-	-	NT	-	-	NT

+ = infected, - = not infected, NT = not tested.

taxonomic keys (Martin, 1987). A diagnostic esterase banding pattern and the ability to induce squash silverleaf disorder (Bedford *et al.,* 1992) identified them as "B" biotype *B. tabaci*.

Insect survival, adaption to host crops and phytotoxic induction

B. tabaci caged on all of the test plants survived significantly longer than those in control cages (Table 2). Sugar beet and the monocotyledons onion and garlic, were poor host plants resulting in high *B. tabaci* mortality and very low colonisation potential. Tomato, broad bean and carrot also caused a high mortality, yet *B. tabaci* were able to colonise these plant species at low levels. All other crop plants tested caused very little mortality and allowed the *B. tabaci* to establish a high level of infestation (Table 2). Phytotoxic responses were caused in a number of the crops ranging from a mild vein clearing in carrots to a severe leaf distortion in lettuce (Table 2).

Virus transmission

The majority of crop varieties tested were not susceptible to infection from any of the viruses. However, turnip (Snowball), tomato (Kondine Red) and French bean (Blue Lake) were susceptible to at least one of the viruses (Table 3). All viruses, except for AGMV, were shown to infect at least one crop species, with AgYVV-s infecting three of the tested varieties.

DISCUSSION

B. tabaci is established in Europe and is spreading to new areas. We have shown that the majority of crop species tested will host "B" biotype *B. tabaci* colonisation and that some crops produce a phytotoxic response to infestation by this insect. However, by far the most serious revelation is that certain European crop cultivars, particularly tomato and French beans, became infected with whitefly-transmitted geminiviruses that are already endemic to other global regions. *B. tabaci* is the vector of not only the six geminiviruses tested in this study, but over 50 other geminiviruses along with several other virus groups (Markham *et al.*, 1994). If European populations of *B. tabaci* gain access to any of these viruses, the possibility must exist that the viruses could be transmitted to plant species within Europe. Since *B. tabaci* has already been found to infest table grape vines in California (Gill, 1992) it is possible that the European viticulture industry is also at some risk. This study shows that there is a need to test major crops and cultivars against potential whitefly-transmitted viruses. Resistant varieties must be identified to enable useful resistance mechanisms to be incorporated within breeding programmes. Weed species that could play important roles as virus reservoirs also need to be identified. We know for example, that nightshade, *Solanum nigrum*, a very common European weed, can be infected by some whitefly-transmitted viruses.

Symptoms, further transmission tests and DNA hybridisations have confirmed that some of the crops used in this study are susceptible to some of the tested viruses. However, it must be stressed that until additional studies are completed, involving further virus transmission passages within these new host plant species, the threat of new endemic virus problems may be spurious. Viruses transmitted to new host plants may in some cases, no longer be acquirable or transmissible by *B. tabaci,* since the virus may be replicating in tissues inaccessible to the insect vector. These "dead-end" host plants have already been identified for some viruses (Markham & Bedford unpublished).

The potential for whitefly-transmitted viruses to survive European winters needs assessment. We already have one such virus which survives overwintering outside in northern Europe, namely honeysuckle yellow vein geminivirus in *Lonicera japonica var. aureo-reticulata*. However, although still infectious by grafting, this virus has been shown to be no longer whitefly-transmissible (Bedford *et al.*, 1992; Bedford *et al.*, 1994). Undoubtably, however, the factor governing virus survival must be the ability of the host plant to overwinter, since experimentally, many of these viruses can be stored in a frozen state for many years (data not shown).

The only recorded transmissible whitefly-transmitted geminivirus found in Europe is in tomato in Italy and Spain. We have shown that more than one whitefly-transmitted geminiviruses will infect tomato, resulting in leaf curl symptoms. At present this European tomato virus is being called tomato yellow leaf curl virus, but until a consensus is achieved for characterising all these different WTGs, its true identity will remain unclear.

Satisfactory control of *B. tabaci* has already proved difficult because of insecticide resistance. Modern control methods must now involve the use of many different strategies in the form of an Integrated Pest Management system (Bedford *et al.*, 1994c). However, once whitefly-transmitted viruses become a problem within a crop, their control by IPM would be very difficult. *B. tabaci* are extremely efficient at transmitting most of these whitefly-transmitted viruses (Bedford *et al.*, 1994a), and only a total eradication of the insect would stop the virus spreading. To combat the threat of these viruses we have to discover the mechanisms involved in their specificity with *B. tabaci* in order to

develop methods of interrupting and breaking the acquisition and transmission cycle.

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DETECTION OF VIRUSES AND INSECTICIDE RESISTANCE IN SUGAR BEET APHIDS CAUGHT IN SUCTION TRAPS

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ABSTRACT

A protocol has been developed which allows individuals of *Macrosiphum euphorbiae* and *Myzus persicae*, caught in suction traps to be tested (using immunoassays) for the presence of the luteoviruses beet mild yellowing virus (BMYV), beet western yellows virus (BWYV) and, for *M. persicae*, the amount of E4/FE4 esterases which gives a measure of this species' resistance to insecticides. From 1991-93 at Broom's Barn and Rothamsted, less than 1% of *M. persicae* and none of the *M. euphorbiae* tested were carrying BMYV; 15-34% of *M. persicae* were carrying BWYV but only one *M. euphorbiae*, at Rothamsted in 1992, was infective with this virus. At both sites, 70-90% of *M. persicae* were typed as susceptible (S) or moderately resistant (R_1) to insecticides. However, over 30% in 1991, 25% in 1992 but only 5% in 1993 were very resistant (R_2) or extremely resistant (R_3). The implications of these results for giving advice on control strategies in sugar beet are discussed.

INTRODUCTION

Virus yellows diseases of sugar beet can reduce potential sugar yields by up to 50%. They are caused by two viruses, the beet mild yellowing luteovirus (BMYV) and the beet yellows closterovirus (BYV). Their control relies on the use of insecticides, either applied at drilling or as foliar sprays, to prevent the build up of the aphid vectors, principally the peach-potato aphid, *Myzus persicae* (Sulz.) but also the potato aphid, *Macrosiphum euphorbiae* (Thos.).

In the U.K. the early-season threshold for applying insecticidal sprays to control virus vectors is one wingless green aphid per four plants (Hull, 1968). However, growers tend to apply sprays as soon as any green aphids are found in the crop, which can result in unnecessary applications because the aphids may not always be carrying virus. To improve the accuracy of advice given, an amplified enzyme-linked immunosorbent assay (ELISA) has been developed to detect BMYV in single aphids (Smith *et al.*, 1991); monoclonal antibodies are used to distinguish between BMYV and the closely related beet western yellows luteovirus (BWYV) which, in Europe, infects

brassica crops including oilseed rape, but rarely sugar beet (Smith & Hinckes, 1985).

An immunoassay which measures the insecticide-degrading esterases (E4/FE4) of individual *M. persicae* (Devonshire *et al.*, 1986), and hence their resistance to insecticides, has enabled annual surveys to be conducted throughout England (Dewar *et al.*, 1988; Smith & Furk, 1989). This information has been used to modify advice given to sugar-beet growers according to the prevalence of the different biotypes. The assay was adapted to allow the analysis of aphids collected in suction traps (Tatchell *et al.*, 1988).

This paper describes an extension of this protocol for the detection of BMYV, BWYV and the resistance to insecticides in individual *M. persicae* collected from the 12.2m suction traps at Rothamsted and Broom's Barn from 1991 to 1993. The results are discussed in relation to improving the virus yellows spray warning scheme for sugar beet.

MATERIALS AND METHODS

Aphid sampling and storage

Between 1991 and 1993 insect samples were collected daily from the 12.2m suction traps at Broom's Barn Experimental Station and Rothamsted Experimental Station. From April until November each year, insects were trapped in a glycerol-based rather than an alcohol-based storage solution (Tatchell *et al.*, 1988) which enabled all *M. persicae* caught to be tested for the presence of virus (Stevens, 1993) and for their level of resistance to insecticides (Devonshire *et al.*, 1986). At Rothamsted, insect samples were sorted immediately in this storage solution, whilst at Broom's Barn samples were transferred to fresh solution and kept at 4°C until being posted to Rothamsted where the aphids were identified and counted.

All alate *M. persicae* and *M. euphorbiae* caught at both sites were removed from the catch, blotted onto absorbent paper, and placed individually in wells of microtitre plates containing 50 µl phosphate-buffered saline with 0.05% Tween 20 (PBS-T). Microtitre plates were stored at -20°C for subsequent assay.

Determination of insecticide resistance

Microtitre plates were allowed to thaw for approximately one hour, the *M. persicae* homogenised using a multihomogeniser and the volume in the wells increased to 250 µl using PBS-T. Twenty-five µl was used for the esterase immunoassay for resistance (Devonshire *et al.*, 1986) and the following threshold absorbance values were used to classify *M. persicae* into broad resistance categories; susceptible (S) A_{620} less than 0.14, moderately resistant (R_1) between 0.14 and 0.6, highly resistant (R_2) between 0.6 and 2.0, and extremely resistant (R_3) greater than A_{620} 2.0. *M. persicae* of known insecticide resistance were used as controls on each plate. *M. euphorbiae* were not tested for insecticide resistance because the immunoassay is specific to *M. persicae*, and there have been no records of resistance in this species to date.

Detection of BMYV and BWYV

The remaining aphid homogenate was divided into two equal parts of 110µl and used to determine the presence of BMYV and BWYV using the amplified-ELISA method (Smith *et al.*, 1991). Monoclonal antibodies, BYDV-PAV-IL-1 (D'Arcy *et al.*, 1989) and MAFF 24 (Stevens, 1993) were used to detect and distinguish between BMYV and the non beet-infecting strains of BWYV. Once the assays were completed the absorbance values were recorded at 490 nm. Aphids were recorded as virus-carriers if readings were above the ELISA absorbance threshold of the mean of six non-viruliferous alate *M. persicae* plus three times their standard deviation. *M. euphorbiae* were tested for BMYV and BWYV in 1992 and 1993 using the above method, except that no homogenate was removed for resistance testing.

RESULTS

In 1991, only two of 196 *M. persicae* caught at Broom's Barn contained BMYV, both of which were moderately resistant to insecticides (R_1 biotypes). Similarly in 1992, only two of 393 *M. persicae* trapped at this site were BMYV-carrying R_1 biotypes. However, none of the 163 *M. persicae* caught in 1993 contained BMYV. None of the *M. persicae* caught at Rothamsted, nor any of the 324 *M. euphorbiae* trapped at the two sites in 1992 and 1993, contained BMYV.

TABLE 1. The proportion of insecticide-resistant biotypes of *Myzus persicae* containing BWYV: 1991 - 1993. (* Total number of aphids caught)

Resistance level	% containing BWYV	No virus	BWYV	No virus	BWYV	No virus
	1991		1992		1993	
Broom's Barn	(196)*		(393)		(163)	
S	2.6	11.7	3.6	22.9	3.7	21.5
R_1	10.7	42.3	8.9	39.7	19.6	50.3
R_2	3.1	21.9	2.0	17.8	0.6	3.7
R_3	0	7.7	0.3	4.8	0	0.6
Total	16.4	83.6	14.8	85.2	23.9	76.1
Rothamsted	(63)*		(232)		(149)	
S	0	23.8	9.0	24.6	10.1	31.5
R_1	12.7	52.4	15.1	40.5	20.8	32.9
R_2	0	9.5	1.3	6.9	2.7	2.0
R_3	0	1.6	0	2.6	0	0
Total	12.7	87.3	25.4	74.6	33.6	66.4

BWYV-carrying *M. persicae* were much more abundant. Between 14.8 and 23.9% of the annual catch of *M. persicae* at Broom's Barn and 12.7 to 33.6% of the aphids caught at Rothamsted carried BWYV (Table 1).

There was no apparent relationship between the presence of BWYV in *M. persicae* and their resistance to insecticides at either site (Table 1). There was a higher proportion of R_2 and R_3 aphids at Broom's Barn in the spring and summer of 1991 but not in 1992 and 1993 (Table 2). There was also a marked increase in proportion of these very resistant biotypes in the autumn of each year at Broom's Barn but not at Rothamsted, although numbers caught in the autumn of 1993 at both sites were small due to cool, wet weather which prevented migration.

One BWYV-carrying *M. euphorbiae* was caught at Rothamsted in 1992, although none were caught the following year. All the *M. euphorbiae* trapped at Broom's Barn were non-viruliferous.

TABLE 2. The number of resistant biotypes of *Myzus persicae* caught in suction traps: 1991-1993.

Year	1991		1992		1993	
	Apr-Jul	Aug-Nov	Apr-Jul	Aug-Nov	Apr-Jul	Aug-Nov
Broom's Barn						
S	9	19	82	22	38	3
R_1	56	49	122	69	106	8
R_2	21	26	7	71	5	2
R_3	6	10	1	19	1	0
Total	92	104	212	181	150	13
Rothamsted						
S	7	8	72	6	60	2
R_1	12	29	121	8	77	3
R_2	4	2	15	4	7	0
R_3	0	1	2	4	0	0
Total	23	40	210	22	144	5

DISCUSSION

This paper describes the exploitation of a protocol for the detection of BMYV, BWYV and the E4/FE4 esterase enzymes in single aphids. With the availability of BYV monoclonal antibodies, as well as other alternative detection systems for this virus, it is anticipated that information concerning the proportion of aphids carrying BYV will also be available in the future, further improving the advice given to the sugar-beet industry.

BMYV-carrying aphids were only caught at Broom's Barn, as might be expected from its location within the main sugar-beet growing region, where there would have been a greater number of virus sources from which aphids could have acquired BMYV. The proportion of BMYV-carrying aphids was very small (<1%), and this equates to the relatively low incidence nationally of virus yellows in the sugar-beet crop; only 3% of plants were infected at the end of August in 1991 and 10% in 1992 and 1993. Any increase in the proportion of BMYV-carrying aphids may indicate the onset of an epidemic year, particularly if virus-carrying aphids are caught early in the season when plants are most susceptible to virus infection. Therefore, it is important to monitor aphid infectivity each year, and, by also determining the proportion of different insecticide-resistant biotypes, the most appropriate control measures can be recommended to help prevent the spread of the disease.

The proportions of resistant variants are broadly in line with recent analyses of populations of *M. persicae* collected from crops (Hockland *et al.*, 1992) with R_1 aphids continuing to predominate, and the more resistant variants accounting for 20-30% of the Broom's Barn populations in 2 of the 3 years, with rather fewer (c. 10%) in the Rothamsted samples. There was a clear trend towards a higher proportion of more resistant variants in the autumn migrants compared with the summer migrants at Broom's Barn, reflecting their likely exposure to insecticides during the growing season in that area.

Approximately 20% of the *M. persicae* caught at Broom's Barn and Rothamsted each year contained BWYV. It is necessary to distinguish between BMYV and the non-beet-infecting strains of BWYV otherwise there could be a serious overestimation of the need for aphicidal sprays which may lead to greater selection for the more resistant biotypes. However, the current data show that there was no relationship between the BWYV content of *M. persicae* and their resistance status. This is not unexpected as there is no known biological reason why aphids of one level of resistance to insecticides should have a greater proportion of virus-carriers than another.

The almost total lack of virus in *M. euphorbiae* in this study contrasts with previous results in which 7% of winged aphids of this species caught in water traps in sugar-beet fields at Broom's Barn carried BMYV and 19% contained BWYV (Smith *et al.*, 1991). These differences may be due to the more selective nature of water traps placed in crops compared to the more random capture of migrant aphids in 12.2 m suction traps (Taylor, 1974). It is important to quantify the importance of *M. euphorbiae* as a vector because it frequently occurs with *M. persicae* in beet crops

especially early in the season, and is difficult to distinguish from that species.

The data on the abundance, timing, infectivity and insecticide resistance status of sugar beet aphids will be used to provide further information to the sugar industry in the regular virus yellows spray warning bulletins issued by Broom's Barn (Dewar, 1994). It is intended that, by collecting these data over a number of years the strategies for the control of the virus vectors can be fine-tuned to maximize profitability for growers and minimize damage to the environment.

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PYMETROZINE, A NOVEL AGENT FOR REDUCING VIRUS TRANSMISSION BY *MYZUS PERSICAE*

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ABSTRACT

Winged individuals of *Myzus persicae* were given access to potato plants infected with potato leaf roll virus (PLRV) for 24 h or potato virus Y^N (PVY^N) for 1 h. They were subsequently transferred to healthy potato plants sprayed 24 h before with 100 mg/l pymetrozine. Pymetrozine reduced transmission of PLRV by 97% and transmission of PVY^N by 75%. The behavioural component of virus transmission was studied with the electrical penetration graph (EPG) technique. Cross experiments were made with infected aphids that probed on treated healthy plants, and uninfected aphids that probed on treated infected plants. The aphids were allowed to probe a known number of cells or to ingest phloem sap for a fixed period. Virus transmission of all individuals was tested with ELISA. Pymetrozine does not effectively inhibit acquisition of PVY^N, but it reduces subsequent transmission to healthy plants by 65%. The results are discussed with respect to the possible regulation of the feeding mechanism in aphids.

INTRODUCTION

Aphids are phytophagous insects with a high reproduction rate mainly based on parthenogenesis, and an efficient dispersion strategy. Few aphicides exist that spare natural enemies and they belong to the traditional insecticide groups. There is a considerable risk that aphicides will eventually become ineffective due to the build-up of insecticide resistance. Hence, there is an urgent need for a potent, safe and selective aphicide which fits in with integrated pest management programs. As some aphid species transmit persistent or non-persistent viruses or both (De Bokx and Piron, 1990; Sylvester, 1989), it would be advantageous if such a selective aphicide could reduce (in some way) the vectoring ability of virus-transmitting aphids. Pymetrozine (CIBA) is a novel insecticide known to be very effective against aphids. Its mode of action is unique as it interferes with the regulatory mechanism of food intake (Kayser *et al.*, 1994).

This paper describes experiments to assess the efficacy of pymetrozine with respect to transmission of the persistent (P) potato leaf roll luteovirus (PLRV) and the non-persistent (NP) necrotic strain of potato Y potyvirus (PVY^N) by *Myzus persicae* (Sulzer), an effective vector of both P and NP potato viruses. PLRV is transmitted from phloem to phloem, whereas PVY^N is present in epidermal and mesophyllic cells. The success of virus transmission was studied using different combinations of pymetrozine-treated and untreated plants with simultaneous monitoring of stylet penetration behaviour.

MATERIALS AND METHODS

Plants and aphids

Potato plants cv. Bintje were grown in a constant temperature glasshouse (20 ± 2°C) in pots of 12 cm diameter. After 3 weeks all stems except one were cut away with a sterilized knife and after transmission experiments were returned to the glasshouse for ELISA tests and visual inspection (± 4 weeks). Stock colonies of an organophosphate-resistant biotype of *M. persicae* (M_3) were reared on Chinese cabbage and L-IV of pre-alatae were selected to complete development on a fresh plant. Unless stated otherwise, winged individuals were used for the experiments.

Plant treatments

Pymetrozine was sprayed onto the plants at a concentration of 100 mg/l, 24 h before the experiments ensuring all leaves were thoroughly wetted. Control plants were sprayed only with water.

Electrical recording of penetration behaviour

The Electrical Penetration Graph (EPG) technique was used as described by Tjallingii (1988). This direct current system has an adjustable voltage source, supplying an electrical potential to the substrate. The equipment was housed in a constant temperature room, isolated from electrical noise and provided with HF fluorescent light and DC-operated spot illumination providing more than 10 000 Lux (120 $\mu E.m^{-2}.s^{-1}$). The aphids after being starved for 2 h were connected to a gold wire of 20 μm diameter with a small droplet of silver paint on the dorsal side of the thorax, just between the implantation of the wings, to which end the aphids were fixed on a vacuum-served operating table. The ground electrode of a DC amplifier with an input resistance of 1 giga Ohm was connected to the pot soil (rooted plants) or water (cut plant parts). The EPG signals were stored directly into a computer through an A/D conversion board and simultaneously, a high frequency recorder (Graphtec WTR 771A) described the patterns on paper charts. Two separate registrations could be followed simultaneously on an oscilloscope. The aphids were lowered onto or removed from a leaf by means of a micromanipulator with electrical control.

Virus transmission experiments

Different procedures were followed in the tests. Aphids were either allowed to feed or probe on infected plants and then to subsequently feed on healthy plants, simulating alighting of aphids coming from outside the crop, or to probe on pymetrozine-treated infected plants, simulating spread of a virus already present in a potato field. Treatments were as follows:

A1) Virus-free aphids were given access to a potato plant infected with PVY. After 1 h they were each transferred to healthy potato plants treated with pymetrozine (Fig.1, A1). After 24 h aphids were removed and the plants placed in a glasshouse for ELISA tests. Controls were transferred onto untreated plants.

A2) Similarly, virus-free aphids were lowered onto a virus-infected plant, but EPG registrations were made and aphids allowed to penetrate 2 or 5 cells (2 and 5 potential

drops (pds)). The experiment was concluded as in A1 (see Fig. 1, A2). Controls to untreated plants again.

B1) Virus-free aphids were lowered onto a cut part of an infected pymetrozine-treated plant and allowed to penetrate 2 or 5 cells as in A2. They were then transferred to healthy, untreated plants (Fig. 1, B1). Controls were as in Fig. 1, A2 left.

B2) Starting as in B1, but aphids transferred to healthy, pymetrozine-treated plants, so they made contact with pymetrozine twice. Controls as in Fig. 1, A2 right.

C1) As in A1, but aphids given access for 24 h to a plant infected with PLRV and transferred to healthy plants treated with pymetrozine for 24 h.

C2) As in C1, but after transfer to a pymetrozine-treated plant the aphids allowed to penetrate into the phloem (E_2-pattern) for 15 min, or for 1 h or until cessation of penetration. Controls in C1 and C2 on untreated plants (continuous feeding).

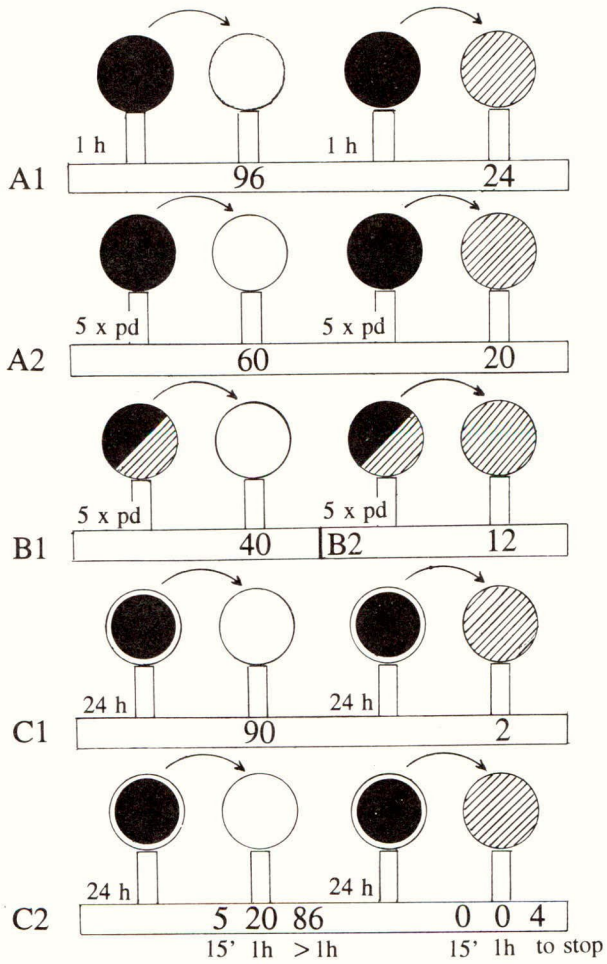

FIGURE 1. Virus transmission experiments. ● plant infected with PVY. ◉ plant infected with PLRV. ○ healthy plant. ▨ healthy plant treated with pymetrozine. Figures within rectangles: % of plants that became infected. Arrows: transfer of aphids.

RESULTS AND DISCUSSION

TABLE 1. Effect of pymetrozine applied to potato plants on penetration behaviour of *Myzus persicae*

Penetration parameters	Untreated	Treated (100 mg/l)	Biological significance
Time to first penetration	1.3 min	1.5 min	direct penetration in both cases
Number of pds to reach the phloem	9.2	9.6	no deterrent effect in cell punctures
Time to reach the phloem at first successful penetration	<20 min	<20 min	no barriers to penetrating the phloem
Longest E_2 pattern	>10 h	<2 h	phloem feeding stops on treated plants
Time to next penetration after phloem feeding	<30 min	>200 min	penetration behaviour disrupted on treated plants
Mortality after 48 h	3 %	88 %	(65 % after 24 h)
Average daily offspring over 48 h	2.3	0.4	reduction in larviposition (reduced feeding)

Fig. 1 shows the proportion of plants that became infected in the different virus transmission experiments. When transferred to untreated plants, the aphids had infected 96% of the plants with NP virus (A1) after 1 h access, which can be expected. The same applies to 90% infection with P-virus after 24 h access (C1). Transmission of both NP and P virus is reduced when the aphids are transferred to treated plants. On plants treated with 100 mg/l of pymetrozine, *M. persicae* starts normal stylet penetration, followed by repeated potential drops (Fig. 2). However, there is no sustained feeding. The E_2 pattern is interrupted within 2 h, after which the aphids withdraw their proboscis caudad and can remain in a non-feeding position for several hours (Table 1). Stylet penetration can then be repeated a few times, to be interrupted after a short period of E_1 or E_2 patterns. The insects usually die within 48 h. The short duration of the E_2 pattern (phloem feeding) explains why inoculation of PLRV is strongly reduced on treated plants but as the onset of penetration is normal, NP viruses could be transmitted.

It may be that the significant reduction in PVY^N transmission as shown in A1 is caused by the limited number of probes in treated plants. Therefore, in A2, only 5 probes occurred on an infected plant and in B1 only 5 probes on an infected, treated plant. Obviously, transmission rate is reduced after a shorter access period and further reduced when the aphids have to inoculate a treated plant. After 2 probes, only 30% of control plants were infected in A2 (not shown).

There was no significant ($P<5\%$) difference in virus acquisition when aphids penetrate into a few cells of a treated plant (B1), but again inoculation into a treated plant is reduced (B2).

We can conclude that penetration of more than a few cells increases acquisition of NP virus in *M. persicae*. Pymetrozine does not affect the number of potential drops (pds) during the first stylet penetration and as a result does not effectively inhibit virus acquisition. However, it interferes with the subsequent transmission to healthy plants. This is because it limits the number of separate probes in treated plants. As penetration of only a few cells is required (Powell, 1991), NP virus transmission cannot be expected to be totally inhibited.

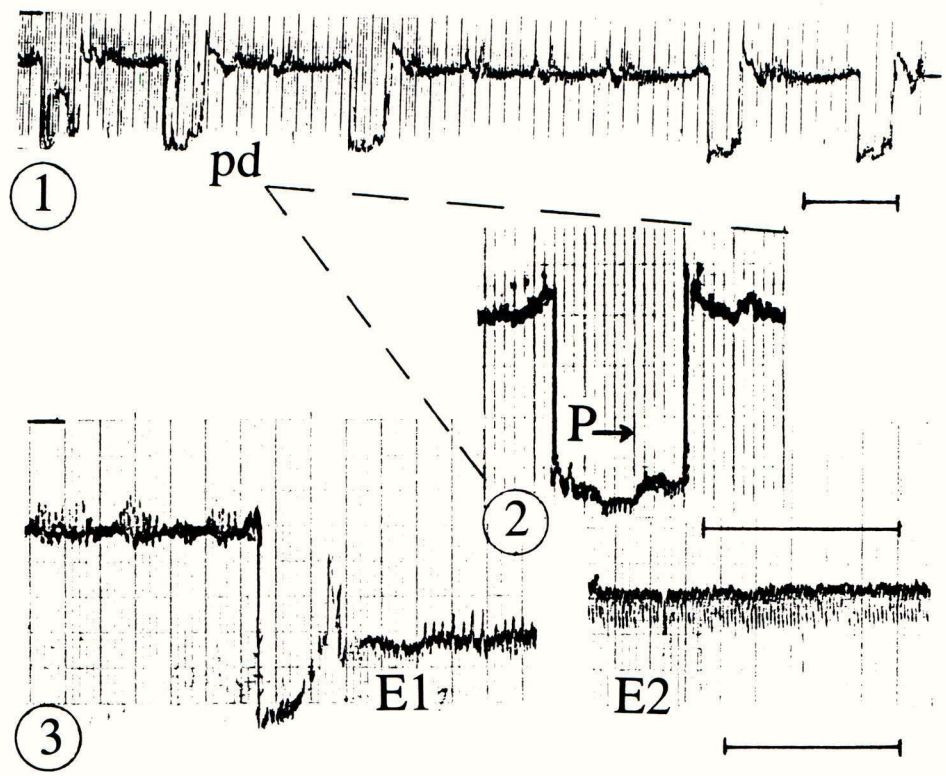

FIGURE 2. EPGs during transmission experiments. (1): 5 subsequent potential drops (pds) before termination of stylet insertion. (2): detail of pd showing signal after puncture of plasmalemma (P→). (3): last pd, stylets enter a phloem cell, followed by E_1, later E_2. Bar (⊢⊣) represents 10 s.

In comparison with A1 only 20 min of phloem feeding gives a lower proportion of PLRV transmission. When access to phloem on treated plants is also limited to 20 min, there is no significant difference in efficiency of PLRV transmission. This means that the effect of pymetrozine on virus transmission of *M. persicae* is largely based on disruption of feeding, after which the aphids stay alive for about the same time as if starved.

Aphids that have been starved for some hours penetrate easily into their hosts in search of water and nutrients. Pymetrozine disrupts normal feeding and inhibits subsequent stylet penetration for a few hours. The repeated sequence of limited sap intake and prolonged starvation eventually kills the aphids and reduces virus transmission. An other behavioural effect of pymetrozine is the reduced mobility of winged aphids that have inbibed the compound after arriving in the crop. In contrast to their reaction after contact with a deterrent allomone, they usually do not try to fly away and hence will not spread any virus on their first feeding site.

The efficiency of transmission of NP viruses by aphids is still under discussion. When these viruses do not enter the food canal, it is difficult to explain how penetrations of more than one cell increase the transmission rate, unless not all cells are infected. According to Powell *et al.* (1992), the frequency with which aphids penetrate cell membranes appears to be related to transmission efficiency. Presence in the food canal may contribute to this phenomenon, and, as Fig. 2 shows, a pd is more than penetration of the plasmalemma alone; a few seconds of fluid uptake may be responsible for the pattern present in the peak of the pds.

As pymetrozine does not affect stylet penetration in the first approach towards the phloem, its effect on NP virus transmission is mainly during the inoculation phase and much less during acquisition. With respect to P virus the strong reduction in transmission rate would seem to be obtained by a modest acquisition together with interrupted phloem feeding during inoculation. When infected aphids arrive from outside the crop, only the inoculation phase is important, but a considerable degree of protection seems still possible.

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