Variation and inheritance of resistance to cypress aphid, *Cinara cupressi* Buckton in *Cupressus lusitanica* Miller

By S M KAMUNYA[1], R K DAY[2], E CHAGALA[3] and P S OLNG'OTIE[4]

[1]*Department of Forestry, Moi University, P O Box 1125 Eldoret, Kenya*
[2]*International Institute of Biological Control, Kenya Station, P O Box 76520, Nairobi, Kenya*
[3]*Kenya Forestry Research Institute, P O Box 20412, Nairobi, Kenya*
[4]*The Tea Research Foundation of Kenya, P O Box 820, Kericho, Kenya*

Summary

Cupressus lusitanica seedlings from open-pollinated seeds of 18 families were inoculated with day-old first instar *Cinara cupressi*. Aphid survival was used to determine the genetic basis and inheritance of resistance to the insect. There was marked variation in aphid survival both between and within families. An individual-tree narrow-sense heritability of 0.76 ± 0.30 shows strong additive genetic control which could allow effective selection and breeding for resistance. Resistant parents produced resistant progeny while susceptible parents produced susceptible progeny. There were notable exceptions as some susceptible parents produced highly resistant progeny, indicating that they had acquired pollen from resistant neighbours. Recovery of aphid damaged trees is evident implying that care should be taken in selecting for resistance. The strong additive variance and potentially high heritability indicate that one cycle of selection may yield a resistant population, while intraspecific crossing may give better results. Implications of the results in a *C. lusitanica* breeding programme are discussed.

Key words: *Cupressus lusitanica, Cinara cupressi,* heritability, resistance

Introduction

The cypress aphid, *Cinara cupressi* Buckton has caused extensive dieback and mortality of *Cupressus lusitanica* Miller. A tree native to Mexico and Guatemala, *C. lusitanica* is widely planted in the East and Central African region on farmlands, in establishment of hedges in rural and urban areas, and is grown in gazetted forests for production of timber. *Cinara cupressi* (Homoptera: Lachnidae) first invaded the African cypress trees in Malawi in 1986 (Chilima, 1991). It then rapidly spread over the East and Central African countries including Tanzania, Burundi, Rwanda, Uganda and Kenya. It also causes considerable damage to species of Cupressaceae in the genera *Callitris, Widdringtonia, Cupressocyparis, Juniperus* and *Thuja*. While it is not a pest in its native Europe, it has rapidly established itself in Africa (Odera, 1991) and Israel (Mendel, 1983) as a most devastating cypress pest.

Colonies of aphids feed on the smaller twigs and branches in the main part of the crown. While feeding they possibly secrete a toxin (Mills, 1990) which causes local chlorosis and branchlet death. Their feeding is accompanied by copious production of honey dew which encourages growth and development of sooty mould resulting in foliage discolouration that may interfere with photosynthesis and gas exchange (Ciesla, 1991). Trees, however, may affect aphids adversely, by influencing their growth rates, survival and fecundity (Memmott, Day & Godfray, 1995). As a result of aphid attack, trees may react by producing allelochemicals which have negative effects on the aphids (Kidd, 1985; Larsson, 1985).

There is a considerable variation in severity of attack on individual trees within affected cypress stands and it is common to find a completely healthy tree adjacent to, and with branches intermingled with, a heavily infested neighbour. This observation suggests that resistance to cypress aphid may be subject to genetic control. The present study investigated the genetic basis of such variation, and recommends methods of developing a population of resistant strains having other desirable silvicultural characteristics.

Materials and Methods

Area of study

The study was conducted at Kenya Forestry Research Institute (KEFRI) and International Institute of Biological Control (IIBC), both of which are located in Muguga, Kiambu District of Kenya. The climate of the area is generally moist and mild with over 954 mm of mean annual rainfall, and a mean annual temperature between 16.4–17.4°C. The landscape consists of undulating to rolling countryside (volcanic foothill ridges) with KEFRI at an altitude of 2095 m above sea level. Soils, developed on tertiary basic igneous rocks (basalts, nepheline phenolites), are well drained, extremely deep, dusky red to reddish brown, friable clay, with acid humic top soil (humic nitosols) (Jaetzold & Schmidt, 1983).

Aphid damage assessment and parent tree selection in seed orchards

Thirty parent trees were selected on 26 July 1994 to represent a range in susceptibility to *C. cupressi*. Resistant and susceptible phenotypes of *C. lusitanica* from Muguga clonal seed orchards (RE 262/67, RE 262/68 and RE 262/69) were selected with the assumption that they are not all escapes and may include resistant genotypes. Damage to the trees by the cypress aphid was scored using a 4 point scale based on the percentage of crown showing the characteristic browning of aphid attack (class 1, 0–10%; class 2, 11–25%; class 3, 26–60%; class 4, 61–100%) (Day *et al.*, 1993).

Trees were scored independently and absolutely, i.e. without relationship to aphid damage level on neighbouring trees. These trees were marked for ease of identification during subsequent seed collection. The selections were done with the constraint that they be separated by at least 50 m. Selected trees, their respective aphid damage categories and the seed orchard in which they belong are shown in Table 1.

Raising of open-pollinated families (half-sibs)

Cones were collected from each of 10 selected trees (based on female parentage) from each of the three Muguga clonal seed orchards on 27 July 1994. They were then dried and the open pollinated seed extracted at Kenya Forestry Seed Centre (KFSC) extraction unit, germinated at a KFSC glass-house and later pricked out into polyethylene pots in which they were raised for 12 months. Two seedlots failed to germinate, probably due to poor viability. There were

28 half-sib families with an average of 80 seedlings per family, out of which 18 families wer₂ put in muslin cages to prevent attack by aphids. Protected seedlings constituted five families each in aphid damage category one and two, and four families in each of aphid damag₂ categories three and four. The protected families were used in the inoculation experiments in ₁ controlled environment room (21–22°C temperature, 50–70% relative humidity and 12:12 ₁ night to day).

Estimation of heritability of resistance to aphid attack on young half-sibs from resistant parents

On attaining 12 months, four *C. lusitanica* seedlings were randomly selected from each of 10 resistant families (parents in aphid damage categories 1 and 2) for survival experiments. The number of seedlings selected per family was dictated by the amount of space available in the controlled environment room. These seedlings were infested with *Cinara* nymphs unde⁰ the environmental conditions stated. The seedlings were first examined carefully for the presence of any aphid or fauna and any found was removed. Two twigs not touching others were selected and a band of sticky non-drying glue was painted near the base of the twigs where they join the main stem, to prevent aphids placed on the twigs from walking off.

From a box of first instar *C. cupressi*, 10 day-old nymphs were transferred onto each of the prepared twigs on the seedlings using a moistened camel hair brush. Inoculated seedlings were then put in the constant environment room in a completely randomised design. The number of aphids alive on each twig was counted on the first, second and then alternate days, up to the 14th day by which time the surviving aphids had reached the adult stage. This experiment was repeated two more times at intervals of one month using new seedlings. Each month represented a block making the whole design a completely randomised block design with three replications of 12-seedling plots (Sokal & Rohlf, 1969).

The nymphs used for inoculation experiments were the progeny of apterous aphids collected as adults from infested *C. lusitanica* plantation at Kamae, Kerita and Uplands, in Kiambu District. The adults were placed in a box with twigs of *C. lusitanica* in the constant environment room, with conditions as mentioned above for 24 h to provide a supply of day-old nymphs for the experiments.

Variation in aphid survival on seedlings from parents in the four damage categories

From each of the four damage categories, nine 12-months old *C. lusitanica* seedlings were selected at random from every family seedling lot. Two twigs from each seedling were selected for the inoculation experiment. Again, 10 day-old aphids were transferred to each twig using the same transfer techniques as in the previous experiments. The number of aphids alive on each twig were counted on the second and then alternate days up to 14th day after inoculation.

Estimation of heritability from parent-progeny regression

An experiment was set up in order to estimate heritability of resistance from parent-progeny regression. This is because aphid damage on *C. lusitanica* in the field could not easily be compared with aphid survival on young seedlings under controlled conditions. Twigs were cut from parent trees in damage categories 1 to 3 and their offspring, and these were used to set up an aphid-survival experiment under similar conditions as earlier described. To justify the use of cut twigs in the experiment, a preliminary investigation involving survival of aphids on attached twigs on 36 seedlings and cut twigs from the same seedlings was conducted. A coefficient of determination of 0.99 arrived at indicated that 99% of aphid survival on attached twigs can be explained by cut twig survival.

Table 1. *Individual patent trees marked for seed collection and their respective aphid damage categories*

Orchard	Category of aphid damage			
	1	2	3	4
RE 262/67	U2	167	169	
	171	160	U5	
	152	K9	206	
		U1		
RE 262/68	T71	197	196	197
	198	183	206	190
	150	192		
RE 262/69	186	186	208	194
	T71	193	189	194
	150	215		

Four twigs were obtained from each of the 14 parents and their progeny respectively. The twigs were about 15 cm long, and their lower portion of foliage was trimmed so they could be stood in a glass tube containing water. The tubes were spaced on trays in such a way that when twigs were placed in them they did not touch each other. The glass tubes were labelled with the tree number from which he twigs were cut.

From a box containing first instar *C. cupressi*, individual nymphs were carefully transferred to the *C. lusitanica* twigs following the procedure described above. Similarly, survival of aphids was monitored as before.

Statistical model and analyses

Estimation of heritability by half-sib analysis

The number of aphids surviving at the end of the experiment (14th day) was converted into percentage and arcsine transformed and the influence of family, trees within family, family × block interaction and blocks examined with a two-way analysis of deviance using GLIM statistical package (Baker, 1987). Individual tree narrow-sense heritability of resistance was estimated as:

$$h^2 = \frac{4\sigma^2_F}{\sigma^2_W + \sigma^2_{BF} + \sigma^2_F}$$

Variance due to various effects were obtained by equating the mean squares to their respective components of variance given in Table 2.

Table 2. *Expectations of mean squares of survival of aphids on 12–14 months* C. lusitanica *half-sibs*

SV	df	MS	Components of variance
Families	F-1	MS_4	$\sigma^2_W + T\sigma^2_{BF} + TB\sigma^2_F$
Blocks	B-1	MS_3	$\sigma^2_W + T\sigma^2_{BF} + TF\sigma^2_R$
Fam. × bl.	(F-1)(B-1)	MS_2	$\sigma^2_W + T\sigma^2_{BF}$
Within plots	BF(T-1)	MS_1	σ^2_W
Total	BFT-1		

F, B and T refer to the families, blocks and trees per family-replication plot.

Table 3. *Analysis of variance of arcsine transformed survival of first instar* Cinara cupressi *nymphs on 14 months old* C. lusitanica *half-sibs*

Source of variation	df	MS	F
Families	9	0.23	4.11**
Blocks	2	0.08	1.37(ns)
Families × Blocks	18	0.05	1.18(ns)
Within-plots	90	0.05	
Total	119		

**$P < 0.01$ and ns not significant at $P = 0.05$

Estimation of heritability by parent-progeny regression

The percentages of aphids alive at the end (day 14) of experiment on each twig were arcsine transformed and a mean calculated for each set of twigs from parents and their progeny respectively. These means were then fitted in a simple regression equation, $Y = bX + e$.

Because the progeny used were half-sib families (i.e. measurements were made only on one parent), the coefficient b is equal to one half the narrow-sense heritability (Wright, 1976; Zobel & Talbert, 1984). Thus $h^2 = 2b$.

Variation in aphid survival on seedlings from parents in the four damage categories

Aphid survival data for the nine seedlings in each damage category were averaged out for the entire experimental period and survival curves plotted. A one-way ANOVA was also carried out to find out whether or not there exists a statistically significant difference in aphid survival at day 14 among the four damage categories.

Results

Estimation of heritability of resistance to aphids in 14 month-old C. lusitanica half-sibs

Table 3 shows that there was a significant ($P < 0.01$) between family difference in survival of aphids, while the block effect (i.e. the three repeats of the experiment) was not significant ($P > 0.05$). The family-block interaction was also not significant ($P > 0.05$) indicating that the survival of aphids was more or less the same among blocks.

Of the total phenotypic variation in aphid survival, 76% is accounted by additive gene effects while 24% may be accounted for by non-additive gene effects and effects other than environmental as the experiment was conducted in a uniform environment. A heritability estimate of 0.76 ± 0.30 is significantly high to indicate genetic differences in resistance to aphid survival. The standard error of heritability is high relative to the estimate. This high standard error is due to high variation between families and limited number of families.

Fig. 1 shows a histogram of the mean percent survival of aphids and corresponding coefficient of variation at the end of the experiment, 14 days after inoculation. The most resistant family is 171 with the least number of surviving aphids, while the most susceptible was 150. One interesting observation is that on family T71. Despite showing the highest aphid survival, its twigs never yellowed or even showed any signs of turning yellow. Some individuals within this family supported aphid survival as high as 80% up to the end of the experiment. This family is therefore very tolerant against the aphid with its tolerance being highly heritable as the mother tree in the orchard also exhibits tremendous tolerance. The
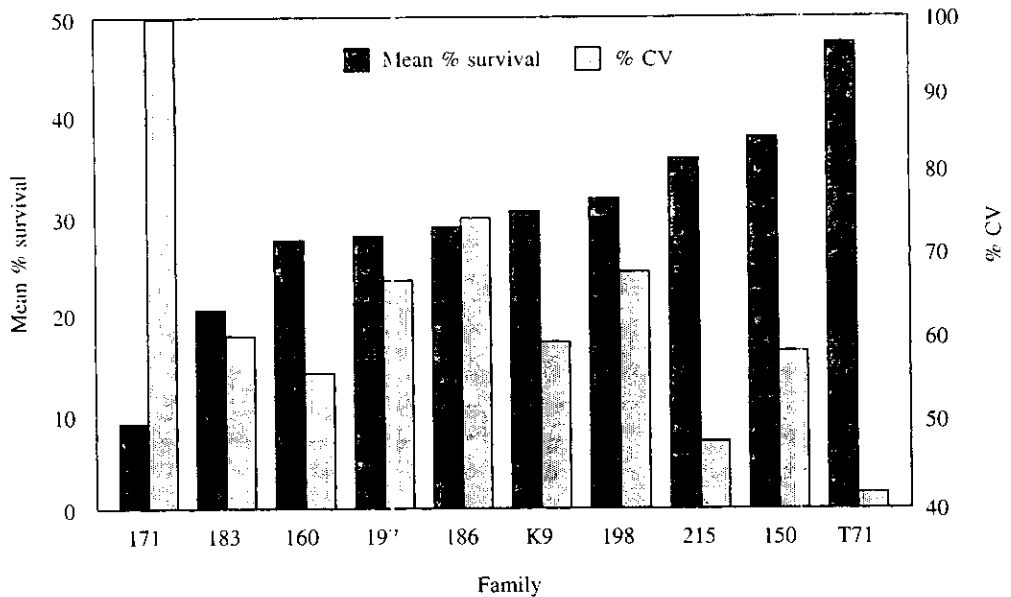
Fig. 1. Aphid survival and respective coefficient of variation on resistant 14-month old *C. lusitanica* half-sib families.

coefficients of variation in aphid survival demonstrate a high degree of heterogeneity in *C. lusitanica* populations with the most resistant family (171) being the most variable.

Variation in survival of aphids on seedlings from parents in different damage categories

The mean survival of aphids on seedlings from parents in different damage categories over the entire experimental period is shown in Fig. 2. Mortality is initially high and then falls as aphids become established and grow older. However, there is wide variation in mortality on seedlings from parents in different damage categories with all aphids dying in some trees.

It is apparent that there is no big difference in survival among seedlings in the first three damage categories, while those in category four showed the highest survival. Analysis of variance at day 14 of aphid survival on seedlings from parents in the four damage categories revealed that survival of aphids on seedlings was significantly influenced by damage categories ($F_{3,32} = 8.96$, $P < 0.01$) to which parents belonged.

Estimation of heritability from parent-progeny regression

The parent-progeny regression yielded a negative regression coefficient ($b = -0.2$), which cannot be used to estimate heritability. An ANOVA constructed for this experiment indicated that the effect due to regression was not significant ($F_{1,12} = 1.28$, $P > 0.05$) implying that the regression coefficient, b is not appropriate to estimate heritability.

Discussion

The present study has revealed considerable variation in resistance to cypress aphid with resistance to be a highly heritable character. The study was carried out in a uniform environment, and therefore phenotypic variance observed could be assumed to be equal to
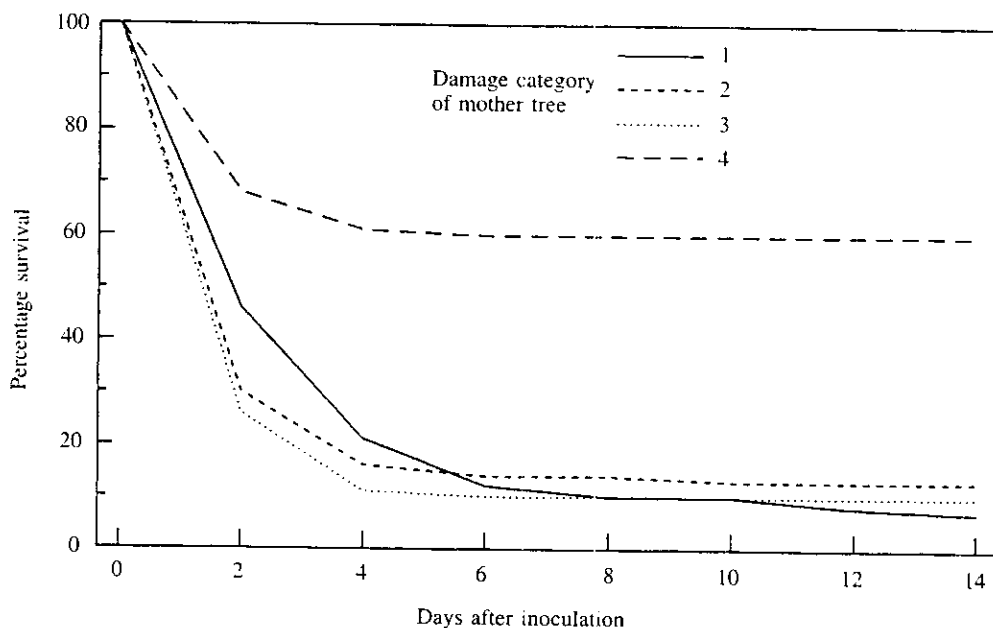
Fig. 2. Survival of aphids on seedlings from parents in different damage categories (DC).

genotypic variance. Individual tree narrow-sense heritability of 0.76 ± 0.30 shows that genetic resistance against aphids is available, with selection expected to give high gains in resistance. The heritability indicates that the additive genetic variance contributes a large proportion of the family variation in resistance. This implies that genetic resistance should easily be obtained if selection and breeding programmes were to be instituted.

The strong additive variance and high heritability of aphid resistance indicate that the straightforward procedure of selecting trees and allowing them to pollinate randomly in a seed orchard would give predictably improved resistance in the offspring. Furthermore, recurrent selection for resistance should effectively accumulate resistant alleles and lead to progressively greater resistance over successive generations of breeding.

The main constraints to achieving economically significant resistance are the usual limitations of restricted selection differentials and perverse genetic correlations that may handicap any breeding programme with multi-trait selection objectives. The problem of restricted selection differential can be avoided by selecting resistant trees from many cypress stands spread over a wide geographical area, or by crossing resistant parents from a large number of stands and selecting progeny prior to testing. Correlation studies reported by Ob ri (1994) showed no significant relationship between aphid damage and growth characters (height and diameter). The lack of correlation between aphid damage to growth characters in *C. lusitanica* could enable breeders to select for resistance in all cypress crops without limitation to tree size.

Heritability is a slightly biased estimate of resistance, in that on average, the number of families used is generally small although the family size is fairly big (12-seedling-plots). But this fact is generally offset by the genetic usefulness of the estimate. Correction of this bias can be done by increasing the number of families to at least 30 and individuals within families to between 10 and 20 (Cotterill & James, 1984; Cotterill, 1990), but this is rarely employed. The foregoing observations notwithstanding, the resulting family variance clearly suggests that resistance is under the influence of the additive gene effects.

The marked variation in survival of aphids on seedlings from parents in the different damage categories reveals a complex inheritance pattern. Such variability in resistance is characteristic of a trait under the influence of additive genes. These progeny, especially those from mothers in damage category one and two must have inherited resistance genes from their mothers and also their fathers to a certain extent. Memmott et al. (1995) attributed such variation of unequal survival of aphids on trees to differences in host plant quality, which they suggested could either be as a result of genetic or microenvironmental causes. The present experiment shows that differences in aphid survival can be attributed to genetic effects.

While inheritance of resistance genes may explain the cause of high mortality in seedlings in damage category one and two, a similar mortality level on seedlings in the third damage category requires a different explaration. More aphids were expected to survive on seedlings from parents in damage category three and four but, surprisingly, survival was quite low for seedlings in damage category three, nearly at the same level as in category one. The reason for this is not readily evident. One possible explanation is that parent trees in damage category three may have received pollen from neighbouring resistant trees and in the process acquired resistant genes. Snow, Mathews, Nance & Foster (1990) found that resistant parents, either male or female, usually produced resistant progeny, and susceptible parents usually produced susceptible offspring. Notable exceptions, however, revealed that one female parent produced highly resistant progeny when crossed with three of the males, but crosses with the other males, including a cross through wind, were highly susceptible. The unknown male parents are likely to introduce uncontrolled variability particularly if the parent tree is pollinated by neighbouring resistant or susceptible trees. This observation is a good indicator that breeding for resistance is a likely alternative or could be undertaken in conjunction with selection in order to obtain resistant populations.

While survival of aphids on twigs from resistant parents is expected to be poor, that of twigs from parents in damage category three was expected to be high. However, the opposite was witnessed. Twigs obtained from their progeny on the other hand recorded a high aphid survival level leading to a negative regression coefficient. The fact that a negative b was noted does not mean that resistance is not under genetic control, and may be strongly influenced by additive genes, as the heritability estimate by half-sib analysis has demonstrated.

It is not clear why a negative b was arrived at but a number of reasons may explain this: a small family size may contribute to this and if families were not from the same population, then the basal population is not in equilibrium which is likely to confound the results (A J Simons, personal communication). Another possible reason is that some trees in higher damage levels might have evolved induced defenses so that any further infestation by the aphids is curtailed (Haukioja, 1990 Kidd, Smith, Lewis & Carter, 1990). Thus trees which earlier had registered a high damage level may later be found to have recovered to a lower damage level. That this might have actually happened was evident during the time of twigs collection from parents in the seed orchard. Some trees which earlier had been placed in damage category three were found to have recovered to lower damage categories. Similar observations were reported by Orondo & Day (1994).

In a study on damage to a cypress stand by cypress aphid, they revealed that the mean damage score for the stand decreased over time. Some trees showed little or no damage throughout the study; many trees showed partial or total recovery, while some trees never recovered and died. One interesting phenomenon centred around aphid feeding behaviour. Once a feeding site turns yellow, aphids were seen moving to or feeding at the greener parts of the branch (twigs) indicating that nutritional characteristics must have significantly changed to the distaste of the aphid. Toxic substances produced by the aphid may be responsible. A similar suggestion has been made by Mills (1990) but no data has been reported to support this. Instances of induced defense have been reported by a number of researchers (Hartley &

Lawton, 1987; Leather, 1990; Kidd *et al.*, 1990). Induced resistance may entail either biochemical or morphological changes. Biochemical changes were detectable in the foliage of birch trees (*Betula pendula* and *B. pubescens*) within hours or days of the leaves being damaged by insect herbivores (Hartley & Lawton, 1990). Within a few hours, phenolic levels increase in artificially and insect damaged leaves of *B. pendula* and *B. pubescens*.

While induced resistance may be advantageous to trees infested by insect pests, it is not heritable, as is constitutive resistance, and therefore cannot be utilised in breeding programmes. Caution is consequently mandatory while planning and carrying out selection and breeding for resistance as selecting trees whose resistance cannot be passed to their offspring may lead to far reaching effects. Trees which have remained consistently and outstandingly resistant need to be identified and only they should be considered in selection and breeding efforts.

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