Original Research Article

A Retrospective Study of *Cinara cupressivora* Damage on *Cupressus lusitanica* Clonal Seed Orchard in Malawi between 1997 and 2003

ABSTRACT

A retrospective study covering a period of seven years (1997-2003) was conducted to assess the extent of damage of *Cinara cupressivora* on *Cupressus lusitanica* Orchard and determine any correlation with climatic factors in Dedza, Malawi. Furthermore, the study was also aimed at determining the efficacy of *Pauesia juniperorum* as a biological control. The data was extracted from monthly reports of the Forestry Research Institute of Malawi (Centre), where a total of 319 trees were assessed. A statistically significant (X^2=13.97, P<0.001) association was found between the damage of the trees and increased number of *Cinara cupressivora*. The damage was found to be eighteen times (Odds Ratio=18.1) more likely to occur on trees attacked by *Cinara cupressivora* than those not attacked. The hot-dry season was found to be significantly (X^2=8.6, P<0.001) associated with the increased number of *Cinara cupressivora*, and the damage was found to be three times (Odds Ratio=3.4) more likely to occur in this season compared to cold-wet and warm-wet seasons. Consequently, the results further shows a significant (X^2=26.37, P<0.001) association between the survival of trees and the presence of *Pauesia juniperorum*. The trees attacked by *Cinara cupressivora* were found to be twenty-nine times (Odds Ratio=29.1) more likely to survive with the presence of *Pauesia juniperorum* than those where the parasitic wasp were absent. It is, therefore, recommended that classical biological control is the most suitable and permanent solution for control. Hence, *Pauesia juniperorum* is a potential agent for the biological control of *Cinara cupressivora*.

Key words: season; biological control; *Pauesia juniperorum*; odds ratio; survive.

1. INTRODUCTION

The cypress aphid, *Cinara cupressivora* is a significant pest of Cupressaceae species and has caused serious damage to naturally regenerating and planted forests in Africa, Europe, Latin America and the Caribbean and the Near East [1, 2]. This indicates that the pest has great dispersal ability and adaptability to different climates and hosts [3]. It was first reported in Africa, from northern Malawi in 1986 [4, 5]. It was then rapidly spread in East and Central African countries, including Burundi, Uganda, Kenya and Tanzania [5, 6]. While it is not a pest in its native Europe and North America, it has rapidly established itself in Africa as a devastating cypress pest [6-8]. By 1991, it was estimated that the aphids is causing an annual loss of growth increment worth $US13.5 [9], and has killed $US 41 million worth of trees in Africa [10] and over $US 2.4 million of losses in Malawi alone [4].

Cinara cupressivora has caused extensive dieback and mortality of *Cupressus lusitanica* Miller. *Cupressus lusitanica* is a tree native to Guatemala and Mexico [11-13]. It is widely planted in Southern, Eastern and Central African region in farmlands as hedges in urban and rural areas and it is grown in gazetted forests for production of timber [6]. *Cinara cupressivora* adults and nymphs suck the plant sap on terminal growth of trees, which causes retarded growth and desiccation of the stems. This may result in a progressive die bark on heavily infested trees. In addition, the aphid feeding is accompanied by copious production of honey dew which encourages the growth of sooty mould. The mould causes foliage discolouration and interferes with photosynthesis and gas exchange [1, 2, 12-16]. The presence of ants and the occurrence of lady bird beetle, which tends to feed on the honey dew is often an indicator of aphid infestation [12, 17, 18].

In view of this, between 1991 and 1993 surveys in Europe and North America were conducted by the International Institute and Biological Control (IIBC) to identify natural control agents to use in Africa. A parasitic wasp *Pauesia juniperorum,* native to Western Europe was finally selected as a potential control agent [19]. *Pauesia juniperorum* only attacks aphids belonging to the sub-family Lachnidae which are found exclusively on conifers. An adult *Pauesia* is about 10 mm long, almost twice the length of its host. It has a black head, brown-black thorax, yellow legs and a yellowish abdomen which becomes darker in older insects. After emerging from its cocoon, the adult female, seeks and lays eggs, inside cypress aphids. Within the parasitized aphid the egg hatches into a larva which feeds and develops rapidly through several instar stages. The parasitoid pupa remains inside the host cuticle which stretches, darkens and becomes hard and gets attached to the twigs. At this stage, the parasitized aphid is referred to as a mummy. The pupal period lasts for about six days. After full development, the adult parasitoid cuts an incomplete circular hole at the posterior end of the mummy to emerge. The total development time from egg to adult is about 14 days. Adults can live up to 7 days [19-24].

In mid and late 1990's consignments of the *Pauesia juniperorum* were released in many *Cupressus lusitanica* hedges and plantations in Malawi. The effectiveness of the parasitoid was observed in the field and a decline in the severity of damage has been observed [21]. However, the quantification of the efficacy of the parasitoid is scarce and the information on the damage caused by the *Cinara cupressivora* and any correlation with climatic factors is limited. Therefore, the objective of this study was to quantify the damage of *Cinara cupressivora* on *Cupressus lusitanica* Orchard and determine any correlation with climatic factors in Dedza, Malawi. The study was also aimed at quantifying the efficacy of *Pauesia juniperorum* as a biological control.

2. MATERIALS AND METHODS

2.1 Study site

The study was conducted in Malawi located in Southern Africa in the tropical savannah region in *Cupressus lusitanica* clonal seed orchard (14^0 19' S; 34^0 15' E and about 1600 m above sea level) at Chongoni Forest Plantation, Dedza. Dedza receives 1200 mm to 1800 mm rainfall per annum, with annual temperature ranging from 7^0C to 25^0C. It is situated about 85 km southeast of the capital Lilongwe. The clonal seed orchard number TB71/2/5 was established in 1971 with twenty one clones from Kenya and each clone had twenty-one laments. The clones were planted in a completely random design in three replicates at a spacing of 3m x 3m.

2.2 Data collection

Records of Biological Control Programme carried out by Forestry Research Institute of Malawi (FRIM) and International Institute and Biological Control for the period starting from January 1997 to September 2003 were used for this study. The records were collected monthly during the stated period and comprised of tree number, clone number, branch number, number of aphids found in the branch, number of *Pauesia juniperorum* or mummy present in the branch, and damage category of the branch. Damage was categorized into two status; live and partially dead. The records comprised of a total of 319 trees.

2.3 Statistical analysis

As data covered a long period of time, care was taken to verify duplication before electronic entry. The collected data was captured onto Microsoft Excel software as a Dbase relational database for editing, validation, verification, and generation of descriptive statistics. Seasonal analysis was examined by dividing the year into three seasons; hot-dry (September-October), warm-wet (November-April), and cold-wet (May-August) seasons. An association between damage and presence of aphids; damage and seasons; and survival of tree or branches and presence of *Pauesia juniperorum* were evaluated by calculating the chi-square (X^2) test for association and the Mantel-Haenszel common odds ratio (OR) at a confidence level of 95% using SPSS Version 17.

3. RESULTS

3.1 Association between Cinara cupressivora and damage on Cupressus lusitanica

Summary of the results on association between *Cinara cupressivora* and damage on *Cupressus lusitanica* are presented in Table 1. The results shows that there were statistically significant (X^2=13.97*, P*<0.001) association between the damage of the trees and increased number of *Cinara cupressivora*. The damage was found to be eighteen times (Odds Ratio=18.1) more likely to occur on trees attacked by *Cinara cupressivora* than those not attacked.

Table 1 Association between presence of *Cinara cupressivora* and damage caused on *Cupressus lusitanica*

		Presence of *Cinara cupressivora* (%)		Total (%)
		Yes	No	
Damage	Partially dead	76.8	16.4	93.2
	Live	1.4	5.4	6.8
	Total	78.2	21.8	100

X^2=13.97, df=1, *P*<0.001, odds ratio=18.1

3.2 Season and presence of Cinara cupressivora

The hot-dry season was found to be significantly (X^2=8.6, *P*<0.001) associated with the increased number of *Cinara cupressivora*, and the damage was found to be three times (Odds Ratio=3.4) more likely to occur in this season compared to cold-wet and warm-wet seasons (Table 2).

Table 2 Mean number of *Cinara cupressivora* present per tree branch during different seasons

Season	Mean number of *Cinara cupressivora* per tree branch±s.e.	Percentage
Hot-dry	5.8±0.4[a]	50.4

Warm-wet	1.8±0.3[c]	15.7
Cold-wet	3.9±0.4[b]	33.9

Note: [a,b,c]Mean with different subscript within a column significantly differ (*P*<0.001)

3.3 Efficacy of Pauesia juniperorum as a biological control on survival of *Cupressus lusitanica* attacked by *Cinara cupressivora*

There were significant (X^2=26.37*, P*<0.001) association between the survival of trees/branches and the presence of *Pauesia juniperorum* (Table 3). The trees/branches attacked by *Cinara cupressivora* were found to be twenty-nine times (Odds Ratio=29.1) more likely to survive with the presence of *Pauesia juniperorum* than those where the parasitic wasp were absent.

Table 3 Association between presence of *Pauesia juniperorum* and survival of *Cupressus lusitanica* attacked by *Cinara cupressivora*

		Presence of *Pausia juniperorum* (%)		Total (%)
		Yes	No	
Damage	Live	85.2	7.4	92.6
	Partially dead	2.1	5.3	7.4
	Total	87.3	12.7	100

X^2=26.37, df=1, *P*<0.001, odds ratio=29.1

4. DISCUSSION

Even though 16.4% of the partially dead trees and branches showed no presence of *Cinara cupressivora*, the finding of this study indicates *Cinara cupressivora* as a major significant risk factor to the damage of *Cupressus lusitanica*. This association is also consistent with the findings in Kenya [13, 23]. According to Orondo and Day [13], the cypress aphid exploits a wide range of feeding sites varying from green branches to woody stems. Damage mainly occurs by sap feeding, as a result the foliage turns yellow and brown. The saliva produced is phototoxic and leads to necrosis in the phloem hence withering of twigs. Feeding retards new growth and causes desiccation of the stems with a progressive dieback of heavily infested trees [10, 12, 13], and this would explain the significant association found in this study. The findings by Owuor [25] also supports the present results. Owuor [25] reported that the overall effect ranges from partial damage to ultimate death of the entire tree and this depends on the infestation severity and in case of severe infestation the death of mature trees can occur within three months.

The study revealed that the peak season for numerous cypress aphids is hot-dry season (September-October) and the population starts build up in cold-wet season (May to August). However, in warm-wet season (November-April; rain season) the population of cypress aphids decline. The significant association of the high occurrence of the cypress aphids in hot-dry season found in this study is consistent with the findings of [26] in Mauritius. According to Alleck et al. [26] the cypress aphids have been reported to increase in number during hot-dry season and decrease in rainy season. For instance, no mummies were observed in a survey in Mauritius during rainy season since the population of the cypress aphid was low [26]. Hot-dry conditions seems to favour both cypress aphid activity and survival [27-32]. A similar pattern was observed in Columbia [33] and Uganda [34]. Hence, cypress aphid populations are strongly influenced by weather conditions, and they are present throughout the year [26, 28, 31]. Findings of a significantly higher number of cypress aphid in hot-dry season in Kenya [13, 23, 35] also support the results of the present study. Mwangi [35] state that, "Population density of cypress aphid is highest during the hot, dry season and lowest during the season of heavy rains. The decline in population density results in some recovery of damaged trees."

The study has revealed that the trees or branches of *Cupressus lusitanica* attacked by *Cinara cupressivora* were found to be twenty-nine times more likely to survive with the presence of *Pausia juniperorum* than those where the parasitic wasp were absent. The present results are in agreement with the results in literature [21-23, 26, 27, 35-38]. According to Chilima and Murphy [21], there is high efficacy of *Pauesia juniperorum* as a biological control on survival of *Cupressus lusitanica* attacked by *Cinara cupressivora* because the female wasp lays its eggs in live cypress aphids and the eggs hatch into larvae that feed on the pest's internal organs, eventually killing it. However, Kairo and Murphy [27] reported that the effectiveness is greater in older hosts, but in young hosts the cypress aphid escape parasitism. Hosts parasitized before they were 9-days-old became mummies without reproducing. Those that were 9 days or older achieved part of their reproductive capacity before dying. Thus, the impact of *Pauesia juniperorum* is markedly reduced when attacking older hosts.

The best effective management option of the aphid is integrated pest management [23, 36]. However, basing on the finding of this study and also, since cypress aphid is an exotic pest, it is, therefore, recommended that classical biological control is the most suitable and permanent solution for control. Hence, *Pauesia juniperorum* is a potential agent for the biological control of *Cinara cupressivora*.

5. CONCLUSION

The present study has revealed that there was statistically significant association between the damage of the trees and increased number of *Cinara cupressivora*. The damage was eighteen times more likely to occur on trees attacked by *Cinara cupressivora* than those not attacked. The hot-dry season was significantly associated with the increased number of *Cinara cupressivora*, and the damage was three times more likely to occur in this season compared to cold-wet and warm-wet seasons. Consequently, the results further shows a significant association between the survival of trees and the presence of *Pauesia juniperorum*. The trees attacked by *Cinara cupressivora* were twenty-nine times more likely to survive with the presence of *Pauesia juniperorum* than those where the parasitic wasp was absent. It is, therefore, recommended that classical biological control is the most suitable and permanent solution for control. Hence, *Pauesia juniperorum* is a potential agent for the biological control of *Cinara cupressivora*.

REFERENCES

1. F.A.O. Forest Pest Species Profile: *Cinara cupressivora* Watson and Voegtlin 1999. FAO, Rome. 2007. (Available at http://www.fao.org/forestry/13557-011835cf4f2791802c40723e174bd99d.pdf)
2. Durak R, Borowiak-Sobkowiak B, Socha M. Bionomy and Ecology of *Cinara cupressi* (Bluckton) (Hemiptera: Aphididae). Polish Journal of Entomology. 2007; 76:107 – 113.
3. Ruiz C, Lanfranco D, Carrillo R, Parra L. Morphometric Variation in the Cypress Aphid *Cinara cupressi* (Bluckton) (Hemiptera: Aphididae) Associated to Urban Trees. Neotropical Entomology. 2014; 43:245 – 251.
4. Chilima CZ. The status and development of conifer aphid damage in Malawi. In: *Exotic Aphid Pests of Conifers. A crisis in African Forestry.* Proceedings of a Workshop at Muguga, Kenya, June 3 – 6, 1991. Food and Agriculture Organisation, Rome, Italy. 1991; 64 – 67.
5. Ciesla WM. Cyprus aphid, Cinara cupressi, a new pest of conifers in eastern and southern Africa. FAO Plant Protection Bulletin. 1991; 39(2/3):82-93.
6. Kamunya SM, Day RK, Chagala E, Olng'otie PS. Variation and inheritance resistance to cypress aphid *Cinara cupressi* Bluckton in *Cupressus lusitanica* Miller. Annals of Applied Biology. 1997; 130:27 – 36.
7. Odera JA. Some opportunities for managing aphids of softwoods plantations in Malawi Assistance to Forestry Sector Malawi, MLW/086/020. FAO, Rome. 1991; pp. 135.

8. Obiri JF. Variation of cypress aphid (*Cinara cupressi*) (Bluckton) attack on the family Cupressaceae. Common Forestry Review. 1994; 73(1):43 – 46.
9. Murphy ST. Cypress severe damaged by the cypress aphid in a rural community in Kenya, Proceedings of a Workshop at Antalya, Turkey, October 13 – 22, 1997, Eleventh World Forestry Congress, 1997.
10. Day RK, Abraham IJ, Kfir R, Murphy ST, Mutitu KE, Chilima CZ. Biological control of homopteran pests of conifers in Africa, *CAB International 2003, Biological control in IPM systems in Africa*. Eds. P. Neuenschwander, C. Borgemeister and J. Langewald. 2003; pp. 101 – 112.
11. Farjon A. A monograph of Cupressaceae and Sciadopitys. Royal Botanical Gardens, Kew. 2005.
12. Memmott J, Day RK, Godfray HCJ. Intraspecific variation in host quality: the aphid *Cinara cupressi* on Mexican cypress, *Cupressus lusitanica*. Ecological Entomology. 1995; 20:153 – 158.
13. Orondo SBO, Day RK. Cypress aphid (*Cinara cupressi*) damage to cypress (*Cupressus lusitanica*) stand in Kenya. International Journal of Pest Management. 1994; 40(2):141 – 144.
14. Normark BB. Molecular systematics and evolution of the aphid family Lachnidae. Molecular Phylogeny. 2000; 14:131-140.
15. Mujtar V, Covelli J, Delfino M, Grau O. Molecular identification of *Cinara cupressi* and *Cinara tujafilina* (Hemiptera, Aphididae). Environmental Entomology. 2009; 38(2):505-512.
16. Foottit RG, Maw HEL, Von Dohlen CD, Hebert PDN. Species identification of aphids (Insecta: Hemiptera: Aphididae) through DNA barcodes. Molecular Ecology Resources. 2008; 8(6):1189-1201.
17. Ciesla WM. *Cinara cupressivora,* NAFC-ExFor. 2003. (Available at www.spfnic.fs.fed.us/exfor/data/pestreports.cfm?pestidval=161&langdisplay=english)
18. Ortiz-Rivas B, Martínez-Torres D. Combination of molecular data support the existence of three main lineages in the phylogeny of aphids (Hemiptera: Aphididae) and the basal position of the subfamily Lachninae. Molecular Phylogenetics and Evolution. 2010; 55(1):305-317.
19. Chilima CZ. Cypress Aphid Control: First African Release of *Pauesia juniperorum.* FRIM Newsletter (Forestry Research Institute of Malawi). 1995; pp. 2.
20. Mutitu KE, Ogembo JO, Chagala E. Rearing *Pauesia juniperorum:* A biological control agent of cypress aphids. KEFRI, Kenya. 2000.

21. Chilima CZ, Murphy ST. The Introduction of *Pauesia juniperorum* (Stary) (Hymenoptere: Bracomidae) into Malawi for the biological control of Cinara species (Homoptera: Aphidae). In: Proceedings of the XXIIUFRO World Congress, Kuala Lumpur, Malaysia, 2000.
22. Kairo MT, Murphy ST. Comparative Studies on populations of *Pauesia juniperorum* (Hymenoptera: Branidae), a biological control agent for *Cinara cupressivora* (Hemiptera: Aphididae). Bulletin of Entomological Research. 2005; 95(6):597 – 603.
23. Mutitu KE. Biological Control of Cypress aphid in Kenya. Tree Pest Management Network Newsletter. 2002; pp. 2.
24. Durak R, Sadowska-Woda I, Machordom A, Borowiak-Sobkowiak B. Biological and genetic studies of Polish populations of *Cinara tujafilina*. Bulletin of Insectology. 2008; 61:159-160.
25. Owuor AL. Exotic conifer aphid in Kenya, their current status and options for management. In: *Exotic Aphid Pests of Conifers. A crisis in African Forestry.* Proceedings of a Workshop at Muguga, Kenya, June 3 – 6, 1991. Food and Agriculture Organisation, Rome, Italy. 1991; pp. 58 – 63.
26. Alleck M, Seewooruthun SI, Ramlugun D. Cypress aphid status in Mauritius and trial releases of Pauesia juniperorum (Hymenoptera: Braconidae), a promising biocontrol agent. MAS 2005, Food and Agricultural Research Council, Reduit, Mauritius. 2005; pp. 313 – 317.
27. Kairo MTK, Murphy ST. Temperature and plant nutrient effects on development survival and reproduction of *Cinara* sp. Nov., an invasive pest of cypress trees in Africa. Entomologia Experiemntalis et Applicata. 1999; 92:147-156.
28. Larsson S. Seasonal Changes in the within-crown distribution of the aphid *Cinara pini*. Oikos. 1985; 45:215 – 222.
29. Robinet C, Roques A. Direct impacts of recent climate warming on insect populations. Integrative Zoology. 2010; 5:132-142.
30. Hulle M, d'Acier AC, Bankhead-Dronnet S, Harrington R. Aphids in the face of global changes. Comptes Rendus Biologies. 2010; 333:497–503.
31. Zhou X, Harrington R, Woiwod I, Perry JN, Bale J, Clark SJ. Effects of temperature on aphid phenology. Global Change Biology. 1995; 1:303-313.
32. Steinkraus D. Factors affecting transmission of fungal pathogens of aphids. Journal of Invertebrate Pathology. 2006; 92:125-131.

33. Watson GW, Voegtlin DJ, Murphy ST, Foottit RG. Biogeography of the *Cinara cupressi* (Hemiptera: Aphididae) on Cupressaceae, with description of a pest species introduced into Africa. Bulletin of Entomological Research. 1999; 89(3):271 – 283.
34. Kiwaso P. Cypress aphid in Uganda. Tree Pest Management Network Newsletter. 2002; pp. 3.
35. Mwangi JG. Integrated Pest Management Model for Kenya. National Cypress Aphid Project, Kenya. 2002.
36. Mills NJ. Biological control of forest aphid pests in Africa. Bulletin of Entomological Research. 1990; 80:31 – 36.
37. Wei JN, Bai BB, Yin TS, Wang Y, Yang Y, Zhao LH, Kuang RP, Xiang RJ. Development and use of parasitoids (Hymenoptera: Aphidiidae and Aphelinidae) for biological control of aphids in China. Biocontrol Science and Technology. 2005; 15(6):533-551.
38. Boettner GH, Elkinton JS, Boettner CJ. Effects of a biological control introduction on three non-target native species of saturniid moths. Conservation Biology. 2000; 14:1798–1806.