Phytochemistry, Vol. 23, No. 8, pp. 1593–1596, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00
Pergamon Press Ltd.

EFFECT OF BIOREGULATOR-TREATED SORGHUM ON GREENBUG FECUNDITY AND FEEDING BEHAVIOR: IMPLICATIONS FOR HOST-PLANT RESISTANCE

DAVID L. DREYER, BRUCE C. CAMPBELL and KENNETH C. JONES

Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture*, Berkeley, California 92710, U.S.A.

(*Received* 9 *August* 1983)

Key Word Index—*Sorghum*; Gramineae; *Schizaphis graminum*; Aphididae; greenbug; plant growth bioregulators; CCC; PIX; host-plant resistance; insect feeding behavior; pectin.

Abstract—Three commercial and six experimental plant growth bioregulators were surveyed for their effect on aphid reproduction when applied to sorghum. Only CCC and PIX had a significant effect on the greenbug, *Schizaphis graminum*. Application of the commercial bioregulators CCC and PIX^{R} caused about a 50% decrease in aphid reproduction rate when applied to greenbug susceptible sorghum but had little effect when applied to a greenbug resistant sorghum line. Electronic monitoring of aphid probing behavior on CCC treated, greenbug-susceptible sorghum showed a response pattern which was indistinguishable from that normally observed on greenbug resistant lines and was different from that associated with aphid probing behavior on untreated susceptible lines. The isolated pectin content of the CCC treated susceptible sorghum was twice that of the controls and had twice the methoxy content. These results support the argument that pectin is a barrier to aphid-stylet penetration for phloem feeding aphids which probe intercellularly and that manipulation of pectin content and/or structure can be a major factor in host-plant resistance to sap-sucking insects.

INTRODUCTION

A number of investigations [1–8] have described the effects of plant-bioregulator treated host-plants on the reproductive rate of aphids (Table 1). Changes in the reproduction rate have been demonstrated for nine different aphid species. This suggests that the chemical effect caused by a given bioregulator on treated plants is general and not limited to any single aphid species. The most widely studied plant bioregulator, effecting fecundity, is CCC (Cycocel^{R}) and, in each case studied, it caused a decrease in aphid reproductive rates when applied to a variety of plant species.

The indirect effects of plant growth bioregulators on insect growth and reproduction has been summarized by Nickell [9]. It has been generally argued that the bioregulators effect aphid reproduction by altering the nutritional quality of the host-plant to the insect. It has been proposed that because of rapid growth and developmental rates, aphids are especially sensitive to subtle dietary changes which can have a pronounced effect on their developmental rates [10]. Thus, a decrease in essential dietary nutrients would be reflected in a slower developmental rate of the aphid. It is argued that since

*Reference to a company and/or product named by the U.S. Department of Agriculture is only for the purpose of information and does not imply approval or recommendation of the product to the exclusion of others which may be suitable.

Table 1. Effect of bioregulator treated plants on aphid reproduction

Aphid species	Host plant	Applied bioregulator	Effect of aphid reproductive rate	Ref.
Acyrthosiphon pisum	Broad beans	2,4-D	increase	1
Acyrthosiphon pisum	Broad beans	maleic hydrazide	decrease	2
Brevicoryne brassicae	Brussel sprouts	CCC	decrease	3, 4, 5
Myzus persicae	Brussel sprouts	CCC	decrease	5, 6
Aphis nerii	Oleander	CCC and Phosphon	decrease	7
Aphis pomi	Apple	CCC	decrease	3
Aphis fabae	Broad beans	CCC	decrease	3
Macrosiphum avenae	Barley	Banvel D, Barban and MCPA	increase	8
Schizaphis graminum	Barley	MCPA	increase	8
Rhopalosiphum padi	Barley	MCPA	increase	8

CCC causes a decrease in the soluble nitrogen levels in treated plants [6, 11, 12] aphids feeding on such plants would incur a nitrogen deficiency. However, in some plants, amino acid content is increased by application of CCC [13, 14] or in others macronutrient levels are not changed [15].

In any case, the above results are based on nitrogen or amino acid analyses of whole plant extracts. Since aphids are largely phloem feeders whole plant nitrogen or amino acid analysis of CCC-treated plants may not have a direct bearing on the above argument since aphids only ingest amino acids translocated in the phloem. Thus, if CCC decreased the rate of translocation of amino acids, then there is less amino acid content available to phloem feeding aphids [3].

The greenbug, *Schizaphis graminum* (Rondani), is an aphid which, on occasion, is a severe pest on sorghum, wheat, barley and some other cereals [16]. This paper reports studies on the effects of bioregulator-treated sorghum on greenbug reproduction and feeding behavior. The work described in this paper was initiated to explore the possibility that plant growth bioregulators might be used as a tool in the study of the chemical basis of aphid–host-plant interactions. If the chemical changes caused by the bioregulator were known or could be determined then the chemical factors which influence aphid feeding might be defined. Finally the results obtained are used to argue the case that middle lamellar pectin is a major barrier to attainment of the phloem during probing and that quantitative and/or qualitative changes in pectin can influence host-plant resistance to sap sucking insects.

RESULTS AND DISCUSSION

Three commercial {CCC [(2-chloroethyl)-trimethylammonium chloride], PIX^R (*N,N*-dimethylpiperidinium chloride) and glyphosate} and six experimental plant bioregulators [17] were applied as a spray to sorghum seedlings. Four weeks later each plant was inoculated with two second-instar greenbugs (biotype C)/plant. After 12 days the number of aphids were counted and the data statistically analysed for differences in the rate of aphid reproduction on treated and control plants. The experimental plant bioregulators and glyphosate treated plants showed no significant effect on the rate of aphid reproduction. On the other hand, CCC and PIX^R treated plants caused a 50% decrease in the rate of aphid reproduction (Table 2)

Table 2. Effect of sorghum treated with 2.5% CCC and 4.2% PIX on aphid reproduction.

	0.3% CCC		4.2% PIX	
Sorghum variety	Control*	Treated*	Control	Treated
BOK 8 (susceptible)	28 ± 3^a	13 ± 3^b	49 ± 7^a	23 ± 4^b
G 449 GBR (resistant)	17 ± 2^{ab}	12 ± 3^b	4 ± 2^c	13 ± 7^{bc}

Number of aphids on bioregular treated and controlled sorghum plants after 12 days.

*Means (±s.e.) followed by different letters within each bioregulator treatment are significantly different ($P < 0.05$).

when applied to an aphid susceptible sorghum line (BOK 8) but showed almost no effect when applied to an aphid resistant line (G 499 GBR).

Previous work in this laboratory [18, 19] showed that some phenolic substances in sorghum and wheat were effective feeding deterrents against the greenbug when incorporated into a synthetic diet. Campbell and coworkers [20] have also demonstrated that biotype C of the greenbug probes intercellularly and is a phloem feeder. If phenolic feeding deterrents were to play a major role in sorghum resistance to the aphid they would have to occur in unrealistically high concentrations in the phloem due to their relatively high ED_{50} values [18]. Moreover, to avoid potential autotoxicity it is likely that most phenolic substances are compartmentalized in vacuoles of plant mesophyll cells which the aphid neatly avoids by its intercellular mode of probing. If phenolic materials did play a deterrent role in aphid resistant sorghum lines then blocking phenolic biosynthesis should lower their concentration and increase the susceptibility of the plant to aphid attack.

When aphid resistant lines were treated with glyphosate, a growth regulator known to block the shikimate biosynthetic route to phenolic and other aromatic substances [21, 22], no difference was observed in the reproductive rate of the aphids applied to control and treated plants. This suggests that the feeding deterrent phenols previously reported from sorghum [18] and wheat [19] do not play a significant role in plant resistance to aphids.

In order to further explore the effect of CCC treated plants on aphid feeding, an electronic monitoring system was used to measure the probing behavior of aphids on CCC treated plants [20]. A number of parameters can be extracted from the electronic monitoring data which give a semiquantitative measure of the acceptability of the plant to the insect. Among these are the total duration of non-probing time (over a 12 hr period), (Table 3) the length of time required by the insect to initially reach the phloem, e.g. duration to the first X-wave (Table 4) and the total duration of phloem ingestion (Table 5).

Aphids feeding on BOK-8 treated with CCC showed a significant decrease in phloem ingestion compared to that on control BOK-8 (Table 5). This reduction in phloem ingestion resembled that of aphids feeding on the resistant G 499 GBR. Furthermore, the duration of non-probing time (Table 3) and duration of time to reach the phloem (time to 1st X-wave, Table 4) were significantly increased for aphids probing the CCC treated BOK-8. In general, the total duration of phloem ingestion is significantly less and non-probing longer on aphid resistant sorghum lines relative to that of susceptible sorghum lines [20]. Furthermore, the reduction in phloem ingestion by

Table 3. Mean total duration of non-probing (min)*

Sorghum variety	Control	0.3% CCC
BOK 8 (susceptible)	28 ± 8^a	40 ± 7^b
G 499 GBR (resistant)	40 ± 10^b	53 ± 26^b

*Means (±s.e.) followed by different letters are significantly different ($P < 0.05$).

Table 4. Length of time required by *S. graminum* to reach the phloem (mean no. of min to first X-wave)*

Sorghum variety	Control	0.3% CCC
BOK 8 (susceptible)	130 ± 19^{a}	186 ± 21^{b}
G 499 GBR (resistant)	209 ± 36^{bc}	252 ± 80^{c}

*Means (±s.e.) followed by different letters are significantly different ($P < 0.05$).

Table 5. Mean total duration of phloem ingestion (min)

	Phloem ingestion	
Sorghum variety	Control	0.3% CCC
BOK 8 (susceptible)	570 ± 20^{a}	469 ± 37^{b}
G 499 GBR (resistant)	395 ± 51^{b}	385 ± 83^{b}

*Means (±s.e.) followed by different letters are significantly different ($P < 0.05$).

greenbugs feeding on naturally resistant varieties is, in part, a result of the longer period taken by the aphids to reach the phloem [23].

The ability of aphids to penetrate the intercellular matrix of plant tissue governs their rate of access to the phloem. The greenbug, in common with many other aphid species which probe intercellularly [24–26], possesses a polygalacturonase [27]. This salivary pectinase depolymerizes the intercellular pectin matrix, facilitating penetration by the stylets of the aphid.

Because the probing behavior of aphids on CCC treated susceptible sorghum was similar to aphids probing untreated resistant sorghum, it suggests that the chemical changes caused by CCC in BOK-8 duplicates the chemical conditions which might occur, naturally, in certain aphid resistant sorghum lines. A case has previously been reported [27] in which natural host plant resistance to the greenbug on sorghum was ascribed to an increased methoxy content of the extractable intercellular pectin. Hence, changes in the concentration or structure of intercellular pectin in a plant should be followed by changes in the rate of depolymerization by the aphid pectinase system and concomittant changes in the duration of time for the aphid to gain access to the phloem.

The measured amounts of extractable pectin and the methoxy content of the pectin from the CCC treated BOK-8 sorghum was doubled relative to that of the controls (Table 6). These results concur with previous results [27] showing that pectin content and/or its chemical nature have a major influence on the rate of aphid stylet penetration to the phloem. The resistant sorghum line (IS 809) previously studied [27] has a different genetic base than the GBR line used in this study. Because the pectin content remained the same and percent methoxy content of the pectin increased only modestly in CCC treated G-499 GBR (Table 6), the mechanism of greenbug resistance of the GBR line used in this study is different than that of IS 809.

Table 6. Yields (% dry wt) and % methoxy content of dewaxed, dried sorghum treated with CCC

	Pectin yield		Pectin % methoxy	
Sorghum variety	Control	0.3% CCC	Control	0.3% CCC
BOK 8	1.4%	3.6%	3.9	8.6
G 499 GBR	2.7%	2.9%	3.9	5.2

It should be noted that there is a large literature on the biochemical changes caused by CCC on wheat, due largely to Blaim and co-workers. Application of CCC as an antilodging agent to wheat seedlings causes an 80% increase in total pectin content [28]. CCC had no effect on protein [29], hemicellulose [29], cellulose [29] content or on the plant's own pectinase [30] activity.

In conclusion, application of CCC to certain crop seedlings could be of further agricultural utility as a means of inducing resistance to aphids or other sap-feeding insects whose mouth-parts penetrate their host-plant intercellularly. This bioinduction could mimic the chemistry which occurs naturally in some resistant sorghum lines.

EXPERIMENTAL

Treatment of plants with bioregulators and measurement of reproduction rate. Seven-day-old seedlings (two leaf stage) were sprayed with an aq. soln of a selected bioregulator containing 0.1% Triton X-100. Control plants received water + Triton X-100 treatment. Ten to 15 replications were run at each concn. After 30 days of growth under greenhouse conditions (16 hr L: 8 hr D photoperiod, about 27°) two second-instar greenbugs (biotype C) were placed on each plant. Aphids were allowed to grow and reproduce for 12 days after which the number of aphids were counted. A second group of plants treated with CCC in the same way, were used to electronically monitor greenbug probing by published methods [20]. In the probing work only one bioregulator concn was used. The concn chosen was that which gave the greatest decrease in the reproduction work. All data were statistically analysed by analysis of variance ($P < 0.05$).

Isolation of pectin. The fresh, chopped plant material was immediately ground in a Waring blender with Me_2CO. Liquid was filtered from the ground plant material and the tissue dried at room temp. The tissue was then treated with 1% HOAc at 100° for 1 hr. The HOAc soln was drained from the tissue by pressing through cheese cloth. Solvent was removed from the filtered soln by freeze drying and the residue precipitated × 2 from water with EtOH. The centrifuged precipitate was dried and weighed. The percent methoxy content was determined by the Ziesel method.

Bioassay of bioregulators as aphid feeding deterrents. In order to show that increased sorghum resistance was not due to a direct effect of CCC or PIX^{R} these substances were added at a series of concns to a synthetic diet and the decrease in greenbug feeding rate measured by previously published procedures [18]. Only at 1% or above was a modest decrease in aphid feeding rate observed. Bhalla and Robinson [31] found that the pea aphid, *Acyrthosiphon pisum*, had a good survival rate on synthetic diets containing CCC but did suffer from reduced fecundity.

Acknowledgements—The authors are indebted to T. E. Mittler, C. B. Montllor, H. Yokoyama and F. Rittig for helpful discussions.

REFERENCES

1. Maxwell, R. C. and Harwood, R. F. (1960) *Ann. Entomol. Soc. Am.* **53**, 199.
2. Robinson, A. G. (1959) *Can. Entomologist* **91**, 527; (1960) **92**, 494.
3. Honeyborne, C. H. B. (1969) *J. Sci. Food Agric.* **20**, 388.
4. van Emden, H. F. (1964) *Nature* **201**, 946.
5. van Emden, H. F. (1969) *Entomol. Exp. Appl.* **12**, 125.
6. van Emden, H. F. (1969) *J. Sci. Food Agric.* **20**, 385.
7. Tahori, A. S., Halevy, A. H., and Zeidler, G. (1965) *J. Sci. Food Agric.* **16**, 568.
8. Hintz, S. D. and Schulz, J. T. (1969) *Proc. N. Cent. Branch ESA* **24**, 114.
9. Nickell, L. G. (1982) *Plant Growth Regulators*, Ch. 13. Springer, Berlin.
10. van Emden, H. F. and Wearing, C. H. (1965) *Ann. Appl. Biol.* **56**, 323.
11. Linser, H., Neumann, K. H. and el Damaty, H. (1965) *Nature* **206**, 893.
12. Yule, W. N., Parups, E. V. and Hoffman, I. (1966) *J. Agric. Food Chem.* **14**, 407.
13. Castro, P. R. C. and Gutierrez, L. E. (1979) *An. Esc. Super Agric. 'Luiz de Queiroz' Univ. Sao Paulo* **36**, 89; (1981) *Chem. Abstr.* **94**, 11590.
14. Gasser, J. K. R. and Thorburn, M. A. P. (1972) *J. Agric. Sci. Camb.* **78**, 393.
15. Castro, P. R. C. (1978) *An. Esc. Super Agric. 'Luiz de Queiroz' Univ. Sao Paulo* **35**, 1; (1980) *Chem. Abstr.* **93**, 199119.
16. Harvey, T. L. and Hackerott, H. L. (1974) *J. Econ. Entomol.* **67**, 377.
17. Yokoyama, H., Hayman, E. P., Hsu, W. J. and Poling, S. M. (1977) *Science* **197**, 1076.
18. Dreyer, D. L., Reese, J. C. and Jones, K. C. (1981) *J. Chem. Ecol.* **7**, 273.
19. Dreyer, D. C. and Jones, K. C. (1981) *Phytochemistry* **20**, 2489.
20. Campbell, B. C., McLean, D. L., Kinsey, M. G., Jones, K. C. and Dreyer, D. L. (1982) *Ent. Exp. Appl.* **31**, 140.
21. Hollaender, H. and Amrhein, N. (1980) *Plant Physiol.* **66**, 823.
22. Amrhein, N., Deus, B., Gehrke, P. and Steinruecken, H. C. (1980) *Plant Physiol.* **66**, 830.
23. Montllor, C. B., Campbell, B. C. and Mittler, T. E. (1983) *Ent. Exp. Appl.* **34**, 99.
24. Adams, J. R. and McAllan, J. W. (1956) *Can. J. Zool.* **34**, 540.
25. McAllan, J. W. and Adams, J. B. (1961) *Can. J. Zool.* **39**, 305.
26. Ehrhardt, P. (1962) *Z. Vergl. Physiol.* **46**, 169.
27. Dreyer, D. L. and Campbell, B. C. (1984) *Experientia* **40**, 24.
28. Blaim, K. and Przeszlakowska, M. (1967) *Bull. Acad. Pol. Sci. (Ser. Sci. Biol.)* **15**, 445.
29. Przeszlakowska, M. (1974) *Acta Agribotanica* **27**, 19.
30. Blaim, K., Przeszlakowska, M. and Szynal, J. (1967) *Zesz. Nauk. Univ. Toruniu Nauki. Mat. Przyr. Biol.* **13**, 211.
31. Bhalla, O. P. and Robinson, A. G. (1968) *Econ. Entomol.* **61**, 552.